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Gutierrez N, Spain
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Hansel JB, Norway
Harrison T, the Netherlands
Hart F, Germany
Hasson E, United Kingdom
Heidelberg M, Switzerland
Hedner J, Sweden
Hengstler G, Germany
Henson R, the Netherlands
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Holter S, Norway
Horvathova M, Czech Republic
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Iolascon A, Italy
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Israel S, Israel
Jansen J, the Netherlands
Janssen J, the Netherlands
Jermias I, Germany
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Johnson P, United Kingdom
Jurczak W, Poland
Karsli S, Sweden
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Kattamis A, Greece
Kiladjian JJ, France
Krause D, Germany
Krisjtinsson S, Sweden
Kroger N, Germany
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Kyrle PA, Austria
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Laperche S, France
Lapidot T, Israel
Leblanc T, France
Lehmann S, Sweden
Lippert E, France
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Lo Celso C, United Kingdom
Lucas O, United Kingdom
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Luminari S, Italy
Macintyre E, France
Maertens J, Belgium
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Medytoth T, Switzerland
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Mesa R, USA
Michallet M, France
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Miccini A, Switzerland
Müller-Tidow C, Germany
Mulligan S, Australia
Munshi N, USA
Muus P, the Netherlands
Narr G, Israel
Nicollin F, France
Nielsen C, Denmark
Nomdedeu J, Spain
Norden A, France
Oakes C, USA
Olarvarrz E, United Kingdom
Oliva E, Italy
Olsson M, Sweden
Oriol A, Spain
Ossenkompele G, the Netherlands
Padea R, France
Papamaniou E, USA
Pellagatti A, United Kingdom
Perez-Simon J, Spain
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Plesner TH, Denmark
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Premawardhena A, Sri Lanka
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Raaijmakers M, the Netherlands
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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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<table>
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<th>Personal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euro</td>
<td>Euro 500</td>
<td>Euro 150</td>
</tr>
</tbody>
</table>

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### Simultaneous sessions I

- New advances in plasma cell disorders and implications for therapy
- Aggressive Non-Hodgkin lymphoma - 1st line
- MRD directed treatment in AML
- New insights into chronic lymphocytic leukemia biology
- Pathogenesis of MDS
- Lymphoma biology
- Thalassemia
- AML: Biology I: Towards molecular therapies
- Hematopoiesis, stem cells and microenvironment
- Gene therapy, cellular immunotherapy and vaccination I

### Presidential Symposium

- Best abstracts

### Poster sessions I

- Acute lymphoblastic leukemia - Biology 1
- Acute lymphoblastic leukemia - Clinical 1
- Acute myeloid leukemia - Biology 2
- Acute myeloid leukemia - Clinical 1
- Acute myeloid leukemia - Clinical 2
- Acute myeloid leukemia - Clinical 3
- Aggressive Non-Hodgkin lymphoma - 1st line
- Bone marrow failure syndromes incl. PNH: Biology
- Chronic lymphocytic leukemia and related disorders - Biology 1
- Chronic lymphocytic leukemia and related disorders - Clinical
- Chronic myeloid leukemia - Clinical 1
- Hematopoiesis, stem cells and microenvironment
- Hodgkin lymphoma
- Iron metabolism, deficiency and overload
- Lymphoma biology
- Multifaced aspects of bleeding disorders
- Myelodysplastic syndromes – Clinical 1
- Myeloma and other monoclonal gammopathies - Biology
- Myeloma and other monoclonal gammopathies – Clinical 1
- Myeloma and other monoclonal gammopathies – Clinical 2
- Myeloproliferative neoplasms – Clinical 1
- Platelet disorders: Basic
- Quality of life, palliative care, ethics and health economics 1
- Stem cell transplantation - Clinical 1
- Thalassemia
- Transfusion medicine
**Simultaneous sessions II**

- Front-line combinations in multiple myeloma and amyloidosis
- Hodgkin and indolent lymphoma - Clinical
- Biology of MPN: JAK2 and beyond
- Clinical trials including treatment discontinuation in CML
- AML: Biology II. Epigenetic targets
- Acquired and inherited platelet disorders
- Acute lymphoblastic leukemia - Biology
- Thrombotic disorders
- Stem cell transplantation - Experimental
- Sickle cell disease, enzymes
- New drugs for rescue in relapsed/refractory multiple myeloma
- Improving prognostication and front-line therapy in chronic lymphocytic leukemia
- Aggressive Non-Hodgkin lymphoma - Relapsed/refractory
- Targeted treatment of AML
- Immunotherapy in ALL
- Biology and disease monitoring in CML
- Prognostic markers and new treatment in MDS
- Stem cell transplantation - Clinical 1
- Bone marrow failure and PNH
- Quality of life, palliative care, ethics and health economics

**Poster sessions II**

- Acute lymphoblastic leukemia - Biology 2
- Acute lymphoblastic leukemia - Clinical 2
- Acute myeloid leukemia - Biology 3
- Acute myeloid leukemia - Biology 4
- Acute myeloid leukemia - Clinical 4
- Acute myeloid leukemia - Clinical 5
- Aggressive Non-Hodgkin lymphoma - Relapsed/refractory
- Bone marrow failure syndromes incl. PNH - Clinical
- Chronic lymphocytic leukemia and related disorders - Biology 2
- Chronic myeloid leukemia - Biology
- Chronic myeloid leukemia - Clinical 2
- Enzymes and sickle cell disease
- Gene therapy, cellular immunotherapy and vaccination
- Indolent Non-Hodgkin lymphoma - Clinical
- Infectious diseases, supportive care
- Myelodysplastic syndromes - Biology
- Myelodysplastic syndromes - Clinical 2
- Myeloma and other monoclonal gammopathies - Clinical 3
- Myeloma and other monoclonal gammopathies - Clinical 4
- Myeloproliferative neoplasms - Biology
- Myeloproliferative neoplasms - Clinical 2
- Other Non-malignant hematopoietic disorders
- Platelet disorders: Clinical
- Quality of life, palliative care, ethics and health economics 2
- Stem cell transplantation - Clinical 2
- Stem cell transplantation - Experimental
- Thrombotic disorders
## TABLE OF CONTENTS

**Simultaneous sessions III**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted therapies in relapsed in chronic lymphocytic leukemia</td>
<td>S769</td>
</tr>
<tr>
<td>Follicular lymphoma - Clinical</td>
<td>S774</td>
</tr>
<tr>
<td>Changing the strategy of therapy in multiple myeloma</td>
<td>S779</td>
</tr>
<tr>
<td>Old and new drugs in MPN</td>
<td>S784</td>
</tr>
<tr>
<td>Childhood and more intensive treatment of AML</td>
<td>S789</td>
</tr>
<tr>
<td>Stem cell transplantation - Clinical 2</td>
<td>S794</td>
</tr>
<tr>
<td>Biomarkers in ALL</td>
<td>S799</td>
</tr>
<tr>
<td>Infectious diseases, supportive care</td>
<td>S804</td>
</tr>
<tr>
<td>Iron: Deficiency and overload</td>
<td>S809</td>
</tr>
<tr>
<td>Gene therapy, cellular immunotherapy and vaccination 2</td>
<td>S814</td>
</tr>
</tbody>
</table>

**E-posters**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoblastic leukemia - Biology</td>
<td>E819</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia - Clinical</td>
<td>E835</td>
</tr>
<tr>
<td>Acute myeloid leukemia - Biology</td>
<td>E864</td>
</tr>
<tr>
<td>Acute myeloid leukemia - Clinical</td>
<td>E906</td>
</tr>
<tr>
<td>Aggressive Non-Hodgkin lymphoma - Clinical</td>
<td>E951</td>
</tr>
<tr>
<td>Bleeding disorders (congenital and acquired)</td>
<td>E974</td>
</tr>
<tr>
<td>Bone marrow failure syndromes incl. PNH - Clinical</td>
<td>E980</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia and related disorders - Biology</td>
<td>E989</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia and related disorders - Clinical</td>
<td>E1016</td>
</tr>
<tr>
<td>Chronic myeloid leukemia - Biology</td>
<td>E1041</td>
</tr>
<tr>
<td>Chronic myeloid leukemia - Clinical</td>
<td>E1074</td>
</tr>
<tr>
<td>Enzymopathies, membranopathies and other anemias</td>
<td>E1078</td>
</tr>
<tr>
<td>Gene therapy, cellular immunotherapy and vaccination</td>
<td>E1099</td>
</tr>
<tr>
<td>Hodgkin lymphoma - Clinical</td>
<td>E1119</td>
</tr>
<tr>
<td>Indolent Non-Hodgkin lymphoma -Clinical</td>
<td>E1128</td>
</tr>
<tr>
<td>Infectious diseases, supportive care</td>
<td>E1143</td>
</tr>
<tr>
<td>Iron metabolism, deficiency and overload</td>
<td>E1154</td>
</tr>
<tr>
<td>Myelodysplastic syndromes - Biology</td>
<td>E1166</td>
</tr>
<tr>
<td>Myelodysplastic syndromes - Clinical</td>
<td>E1179</td>
</tr>
<tr>
<td>Myeloma and other monoclonal gammopathies - Biology</td>
<td>E1200</td>
</tr>
<tr>
<td>Myeloma and other monoclonal gammopathies - Clinical</td>
<td>E1259</td>
</tr>
<tr>
<td>Myeloproliferative neoplasms - Biology</td>
<td>E1307</td>
</tr>
<tr>
<td>Myeloproliferative neoplasms - Clinical</td>
<td>E1320</td>
</tr>
<tr>
<td>Non-Hodgkin &amp; Hodgkin lymphoma - Biology</td>
<td>E1352</td>
</tr>
<tr>
<td>Other Non-malignant hematopoietic disorders</td>
<td>E1410</td>
</tr>
<tr>
<td>Platelets disorders</td>
<td>E1430</td>
</tr>
<tr>
<td>Quality of life, palliative care, ethics and health economics</td>
<td>E1457</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>E1481</td>
</tr>
<tr>
<td>Stem cell transplantation - Clinical</td>
<td>E1496</td>
</tr>
<tr>
<td>Stem cell transplantation - Experimental</td>
<td>E1556</td>
</tr>
<tr>
<td>Thalassemias</td>
<td>E1570</td>
</tr>
<tr>
<td>Thrombosis and vascular biology</td>
<td>E1590</td>
</tr>
<tr>
<td>Transfusion medicine</td>
<td>E1605</td>
</tr>
</tbody>
</table>

*Note: Pages vary from 301 to 651.*
TABLE OF CONTENTS

Publication Only

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
Quality of life, palliative care, ethics and health economics
Sickle cell disease
Stem cell transplantation - Clinical
Thalassemias
Thrombosis and vascular biology
Transfusion medicine

Late Breaking Oral Session

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
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: Cyto genetic risk status in multiple myeloma (MM) studies is tradition ally 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 cyto genetic 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 WWVOX, and for 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, cyto genetic risk status in the CASTOR and POLLUX studies was defined as high risk with either t(4;14), t(14;14), 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).

Table 1.

<table>
<thead>
<tr>
<th>Concordance rate between FISH and NGS</th>
<th>POLLUX</th>
<th>CASTOR</th>
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</thead>
<tbody>
<tr>
<td>t(4;14)</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>100%</td>
<td>97%</td>
</tr>
<tr>
<td>del17p</td>
<td>100%</td>
<td>98%</td>
</tr>
</tbody>
</table>

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 cyto genetic risk in patients with RRMM. The utility of NGS in these clinical studies extends far beyond the detection of cyto genetic 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 status 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 (10−4, 10−5, and 10−6) 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=0.04). 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=0.0011) 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 64% 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 EMN02/HO95 PHASE 3 TRIAL**

**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 EMN02/HO95 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 Bruinink 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 del17, t(4;14) or t(14;16); 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 MFC 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 MDR-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 MDR-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 MDR positive patients at pre-maintenance who had a second MDR evaluation after at least 1 year of lenalidomide became MDR-negative.

**Summary/Conclusions:** MFC by MRD 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 MDR-positive patients.

**S103**

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

**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 antigen for CAR T-cell therapies in multiple myeloma.

**Aims:** This phase I, open-label trial was conducted to assess the efficacy and
Patients with Light Chain Amyloidosis Treated with NEO001 Achieve Rapid Organ Responses that are Independent of Previous Plasma Cell–Directed Therapies


Aims: To assess the association between responses and time, 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/VGPR/PR after PCD: 47.1/66.7/42.9% [cardiac] and 68.8/63.6/62.5% [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. The nodules were obviously decreased after the infusion and disappeared finally. Another one achieved transient partial response, which last for 12 days.

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.
Background: Mantle cell lymphoma (MCL) is currently an incurable disease. In spite of high complete response rates (CR) after initial immunochemotherapy induction followed by autologous stem cell transplantation (ASCT), MCL patients experience iterative relapses.

Aims: We investigated whether or not rituximab maintenance (RM; 375mg/m2 every 3 months for 3 years) after ASCT prolongs response duration.

Methods: This phase III trial included 299 patients (65+6) at diagnosis, of whom 240 were randomly assigned to RM or observation after ASCT. The primary end point was event-free survival (EFS) (progression, relapse, death, severe infection during RM) after ASCT.

Results: After 4 courses of immunochemotherapy induction (R-DHAP; Rituximab, dexamethasone, cytarabine, platinum derivative), overall response and CR rates were 89.3% and 77.3%, respectively. ASCT was performed in 257 patients. Median follow-up from randomization after ASCT was 50.2 (46.4-54.2) months. Starting from randomization, 4-year EFS was 78.9% (95%CI; 69.8 to 87.0) for RM and 56.2% (95%CI; 45.7 to 66.7) for observation (n=120) (p=0.001, 4-year progression-free survival (PFS) was 82.2% (95% CI; 73.2 to 88.4) for RM versus 64.6% (95% CI; 54.6 to 73.0) for observation (p=0.0005) and OS was 88.7% (95% CI; 80.7 to 93.5) for RM versus 81.4% (95% CI; 72.3 to 87.7) for observation (p=0.0413). The death rate was lower for patients in the RM arm (HR=0.95; 95% CI; 0.255 to 0.986) than for patients in the observation arm.

Summary/Conclusions: The LyMa trial demonstrates for the first time that ASCT after R-DHAP prolongs EFS, PFS and OS. Thus, 4 courses of R-DHAP plus ASCT (without TBI) followed by RM maintenance (one infusion every 3 months for 2 years) is a new standard of care for young MCL patients.
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>Ratiobased SC plus CHOP</th>
<th>Ratiobased IV plus CHOP</th>
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<tbody>
<tr>
<td>N (%) / % of patients</td>
<td>N (%) / % of patients</td>
<td></td>
</tr>
<tr>
<td>Fair or Good response</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>70.6 (42.3–55.9)</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>42.4 (34.0–50.7)</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>32.8 (26.7–38.8)</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>32.8 (26.8–38.1)</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Progression rate</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>14.0 (6.4–21.9)</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>14.3 (6.1–21.9)</td>
<td>81</td>
<td></td>
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<tr>
<td>High risk modified NOS</td>
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<td>342</td>
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<tr>
<td>62.3 (37.8–86.8)</td>
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<tr>
<td>74.8 (46.1–93.8)</td>
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<tr>
<td>High risk not modified NOS</td>
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<td>342</td>
</tr>
<tr>
<td>61.0 (26.5–87.5)</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>71.3 (57.8–89.1)</td>
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<td>High risk del CDKN2A</td>
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<tr>
<td>61.0 (26.5–87.5)</td>
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<td></td>
</tr>
<tr>
<td>61.0 (26.5–87.5)</td>
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<td>High risk del TP53</td>
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<tr>
<td>87.4 (32.0–97.0)</td>
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<tr>
<td>87.4 (32.0–97.1)</td>
<td>81</td>
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</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.

S109

ANALYSIS AND CHARACTERIZATION OF HEMATOLOGIC CANCERS USING A COMPREHENSIVE NGS PANEL COMPRISING 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 neoplasm genomes. 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-XKR3 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 break-point coordinator.

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, 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.

S108

TP53 MUTATIONS, BUT NOT DELETION OF TP53 AND CDKN2A, HAVE INDEPENDENT PROGNOSTIC VALUE IN MANTLE CELL LYMPHOMA 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 and <66 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, 5.5 and 10.2 years, respectively. In our mutational analyses (n=147), only TP53 had prognostic impact in multivariate analyses (MVAs). 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 mutations in 29 patients (13%) revealed 14 (48%) with TP53 in 38 (22%). Eight patients carried both deletions. Del-CDKN2A was significantly 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, Ki67-index>30%, NOTCH1 mutations, 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 poorer 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

S110

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


Background: Mutations in Fms-like tyrosine kinase 3 (FLT3) are common in patients with acute myeloid leukemia (AML) and are associated with an aggressive disease course and a poor prognosis. Notably, FLT3 internal tandem duplications (ITD) predict early relapse and short overall survival (OS) after chemotherapy. Gilteritinib, a highly selective FLT3/AXL inhibitor, has displayed antileukemic activity in FLT3 mutation-positive (FLT3mut+) relapsed/refractory (R/R) AML in the CHRYSLIS Phase 1/2 study (NCT02014558), specifically at doses ≥80 mg/d. In FLT3-ITDmut+R/R AML correlated with clinical response and 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.

Methods: This exploratory analysis evaluated molecular response in patients aged ≥18 years with FLT3mut+/R/R AML who had received treatment with 120 or 200 mg/d gilteritinib. These doses were identified due to their ability to induce high clinical response rates, and consistent, potent FLT3 inhibition in correlative assays. Molecular response was assessed in patients who 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. 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:

MRD negative 15 417 (224-248) 67 213 (144-246) 0.02
MRD positive 20 417 (248-334) 40 99 (142-234) <0.01

Table 1. Overall survival in subjects who achieved a molecular response compared with those who did not by depth of response.
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 ALOLOGENIC 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 after 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=282), or conventional PRT by a third cycle 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 covariate analysis was performed by applying multivariable Cox regression with MRD as primary endpoint.

Results: MRD was positive in 129 (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 (65±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±2%. NRM split by EBMT risk score showed less NRM in patients with a low EBMT-risk score as compared to patients with a high EBMT risk score (s2 compared to >s2, 10±2% compared to 22±4%, p<0.005, respectively). Multivariable analysis with adjustment for covariates showed that the incidence of relapse was significantly reduced following alloHSCT as compared to chemotherapy or autologous HSCT (HR 0.36, p<0.001), which was similarly exerted in MRD negative and positive patients (HR 0.38, p<0.001 and HR 0.35, p<0.001). RFS was also improved following alloHSCT as compared to chemotherapy or autologous HSCT (HR 0.53, p<0.001), while no significant differences were found for OS (Figure 1).

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-SAKK H102 trial to prospectively define, using flow cytometry, the leukemic CD34+CD38- stem cell frequency combined with MRD frequencies 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-positive cells above 1% (as compared to total amount of WBCs) and LSC-positivity was defined as a CD34+CD38-LSC percentage above 0.00001% (LSC cut-off 0.00001%); thus no CD34+CD38-LSC events measured.

Results: Cumulative incidence of relapse (CIR) and overall survival (OS) data were investigated for four different MRD/LSC groups: 1. MRDpos+LSCneg patients (n=116) 2. MRDpos+LSCneg patients (n=28) 3. MRDneg+LSCpos patients (n=58) and 4. MRDpos+LSCpos patients (n=20). Results showed that MRDpos+LSCneg patients have the worst prognosis. 3-year CIR for the four above-defined groups was 35% (SE 4), 43% (SE 9), 53% (SE 7), and 100% (SE 0), respectively. Similar results were found for OS: 3-year OS was 66% (SE 4), 68% (SE 9), 53% (SE 8), and 100%, respectively, with 17 patients dead and 3 censored in the latter group. When investigating the impact of MRD/LSC status in the good, intermediate, poor and very poor risk group (according to HOVON), patient numbers were sometimes small; however, results show that MRDpos+LSCposAML patients in all different risk categories have a very poor prognosis. Moreover, multivariate analyses, containing all well known risk factors including risk group and post remission treatment, showed that MRDpos+LSCpos patients have a significantly worse cumulative incidence of relapse (hazard ratio [HR] 5.89; 95% CI 3.32-10.47) and overall survival (HR 3.62; 95% CI 1.86-7.04) as compared to the MRDneg+LSCneg patient group.

Summary/Conclusions: Overall, we conclude that our prospective results show that CD34+CD38-LSC frequency has important additional value in MRD assessment and that it especially enables to identify very poor risk patients in...
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.

S114
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+ were 63% vs 44% vs 37% vs 25% for all patients; 69% vs 51% vs 50% vs 30% excluding poor-risk patients and 76% 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 NPM1wt 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 NPM1wt standard risk patients with 5 year OS of 32% vs 64% (P=0.002) for MRD+ vs MRD- (Figure 1). 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.
New insights into chronic lymphocytic leukemia biology

S115

CLINICAL IMPACT OF THE SUBCLONAL ARCHITECTURE AND MUTATIONAL COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Recent studies have revealed the presence and prognostic impact of small mutated subclones in chronic lymphocytic leukemia (CLL) (Rossi et al 2014, Nadeu et al 2016, Rasi et al 2016). Although these studies focused only on a small subset of 5 genes, their results opened a new perspective where the proportion of cells carrying each specific driver mutation may contribute to the evolution of this disease. Moreover, the subclonal and mutational complexity estimated by the presence of subclonal driver alterations (Landau et al 2013, Landau et al 2015) or the accumulation of driver mutations (Puente et al 2015) have been proposed as promising indicators of clinical behavior.

Aims: The goal of this study was to determine the relevance of the quantitative subclonal architecture and mutational complexity in the evolution of CLL integrating the deep sequencing analysis of a large panel of driver genes and DNA copy number alterations (CNA).

Methods: The mutational status of 28 driver genes was investigated in 406 previously untreated CLL patients by targeted next-generation sequencing (NGS). Mutations present in less than 1% of tumor cells were identified. All low frequency mutations were verified by allele-specific PCR or a second round of NGS. CNAs were analyzed by SNP-arrays. Alterations were classified as clonal if their CCF was ≥85%, and subclonal otherwise. All patients gave informed consent.

Results: Using a highly sensitive NGS strategy we observed that small subclonal mutations were the sole alteration in 22% of the mutated cases, and were frequently detected in nearly all investigated genes. We identified three gene-specific patterns that linked the magnitude of the mutated clones (or mutated cancer cell fraction, CCF) with the prognosis of the patients: i) CCF-independent pattern: mutations at any CCF had prognostic value, ii) CCF-gradient pattern: the poor prognostic impact was a continuous variable directly related to the size of the clone, and iii) CCF-clonal pattern: only mutations with a CCF above a certain threshold impacted the outcome of the patients. Combining mutations and driver CNAs of the panel of the previously identified subclonal driver alterations in 66%. On the other hand, subclonal driver alterations were present in 60% of the patients. The mutational complexity (accumulation of 1 to 4x driver alterations), but not the presence of subclonal driver populations, gradually shortened the time to first treatment independently of the IGHV mutational status and Binet stage. Conversely, the subclonal complexity, defined as the accumulation of driver alterations with the presence of at least one driver subclone, predicted for a worse overall survival independently of the IGHV and Binet stage. Patients with a pure clonal population (presence of one or more driver alterations in all tumor cells) had a similar overall survival than patients without any alteration.

Summary/Conclusions: Our study shows that the prognostic impact of different driver mutations is related to the size of the mutated population. Therefore, the clinical evaluation of gene mutations should consider the quantitative representation of the mutations and not only their presence or absence. In addition, the mutational complexity predicts for shorter time to first treatment independently of the IGHV and Binet stage, whereas the subclonal complexity confers an independent adverse impact for overall survival. Altogether, the integration of the subclonal architecture and mutational complexity in prognostic indexes may improve the stratification of CLL patients.

S116

FBXW7 MUTATIONS LEAD TO ACCUMULATION OF NOTCH1, HIF1-ALPHA 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, HIF-1-alpha and c-MYC and thereby targets them for proteasomal degradation.

Aims: 1) To study the functional consequences of FBXW7 mutations. Approximately 60% of FBXW7 mutations alter arginine residues that are involved in substrate targeting. In T-cell acute lymphoblastic leukemia these arginine mutations are associated with chemotherapeutical resistance. In CLL however, the role of dysfunctional FBXW7 is unclear. We therefore aimed to delineate the prevalence of FBXW7 mutations in CLL patient cohorts and characterize its functional role. 2) To examine the role of mutations in the PolyPhen-2 software. All except one missense mutation in FBXW7 were predicted to be most likely damaging. No mutations in FBXW7 were found in the CLC, MCL, and CLL cell lines analyzed. To determine the functional consequence of FBXW7 mutations in CLL, we induced either a heterozygous or a homozygous truncation of FBXW7 in the CLL cell line HG3, resulting in the loss of the substrate binding site of the WD40 domain. The homozygous truncation of FBXW7 resulted in an increase of NOTCH1, HIF1-alpha, and c-MYC protein levels, whereas no difference of Cyclin E protein amount was detectable. In addition, an elevation of NOTCH1 activity was found in both the heterozygously and homozygously truncated mutant cell lines in comparison to the wildtype HG3 cell line. To confirm this finding, protein levels of 5 CLL patients with FBXW7 mutations were analyzed with a similar outcome.

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 therefore leads 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.
of CLL in relation to the mutational, transcriptional and three-dimensional (3D) chromatin landscape.

Methods: Seven CLL patients with distinct clinico-pathological features and five mature B-cell subpopulations were extensively analysed using (i) ChIP-seq of six different histone marks with non-overlapping features (H3K27ac, H3K4me1, H3K4me3, H3K9me3, H3K27me3 and H3K36me3); (ii) single stranded RNA-seq; (iii) transposable-accessible chromatin assays (ATAC-seq) and iv) whole-genome bisulphite sequencing (WGBS), creating a unique reference epigenome for CLL. These data were complemented with the 3D chromatin landscape in one CLL case measured by high-throughput chromatin conformation capture (HiC-seq) and promoter capture Hi-C (PChi-C). Furthermore, we mapped the active chromatin landscape of 100 CLL patients by H3K27ac ChIP-seq and ATAC-seq. Whole-genome sequencing data was available for 44 of these patients. We applied a broad range of bioinformatic tools to analyze the data in an integrative way.

Results: CLL is distinct from normal B cells for all layers of the reference epigenome (e.g. IGVH), and the active chromatin landscape in CLL is less differentiated than that in normal B cells. CLL cell lines are more closely related to naive memory B cells than to germinal center B cells and plasma cells. Interestingly, in CLL we not only saw activation of regions that are active in naive and memory B cells, but also an unexpected activation of genomic regions that are specifically active in germinal center B cells and plasma cells. Changes in activation in these and other regions could furthermore distinguish the two major clinical subgroups of CLL with unmutated and mutated immunoglobulin heavy chains (IGVH). CLLs did not only differ from normal B cells regarding the separate layers of information, but also using combined patterns of histone marks, which for example can define regulatory elements as active promoters (H3K4me3 and H3K27ac) or active enhancers (H3K27ac and H3K4me1). More specifically, we detected 534 genomic regions with de novo gain (n=498) or loss (n=36) of active regulatory regions in CLL. Large regions (>10kb) showing de novo gain of regulatory elements in CLL (n=51), were located into, close to, or interacted in 3D space with genes important for CLL pathogenesis, e.g., LEF1, BCL2 and FMOD. Interestingly, non-coding somatic mutations in IGVH mutated CLLs accumulate in these and other active regulatory regions, likely off-target effects of the somatic hypermutation machinery. Besides changes in regulatory elements, we observed that CLLs lost poised promoters, which are replaced by repressive active regions. This change, mainly occurring in developmental genes, does not affect gene expression levels, as these genes are already silent in normal B cells. It may however represent loss of plasticity during CLL pathogenesis in which these genes become permanently inactive.

Summary/Conclusions: With this integrative study, we generated new conceptual avenues to understand the complex link among the epigenetic, mutational, transcriptional and 3D chromatin landscape in CLL. In addition we provide the community with an extensive resource of epigenetic information of this lymphoid neoplasm.

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

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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 unclear.

Aims: We explored the mechanistic underpinnings of CLL cell survival in response to BAFF signaling.

Methods: We established a novel BAFF-expressing stromal co-culture model and dosed inhibitors of Bruton tyrosine kinase (BTK), ibrutinib, phosphoinoside-3 kinase (PI3K, idelalisib) and spleen tyrosine kinase (SYK, entosple tinib). We quantified CLL cell apoptosis, migration, NFκB activity, protein and mRNA expression by flow cytometry, immunoblotting, ELISA, RT-PCR and immunocytochemistry.

Results: CLL cells co-cultured with BAFF-expressing stroma were resistant to spontaneous apoptosis (12.3±3.2% after 24 h, vs 34.8±6.2% off stroma) and chemotherapy agents (bendamustine, fludarabine). Gene expression profiling exposed the NFκB pathway gene targets as the most significantly upregulated upon BAFF stimulation (p<0.0001). We and others have shown that BAFF-expressing stroma induces canonical and non-canonical NFκB in CLL. By contrast, while BAFF led to strong activation of the non-canonical NFκB with processing of p100 (to p52) by 4 h and a 5-fold increase in p52 DNA-binding activity by 24 h, canonical NFκB (RelA) activation was less pronounced. BAFF predominantly induced Mcl-1, compared to CD40L which strongly upregulated Bcl-X. BCR is a major driver of canonical NFκB signaling in CLL. Thus, we studied whether BAFF co-opted BCR signaling in CLL. BAFF induced rapid (15 min) phosphorylation of the proximal BCR kinases SYK and LYN, sustained for up to 4 h, as well as ERK, in CLL cells. AKT acti- vation occurred late (>2h), suggesting that BAFF induced AKT independent of BCR. BAFF-mediated BCR activation did not correlate with IGVH muta- tional status. Like IgM, BAFF induced CLL cell chemotaxis. SYK inhibition effectively antagonized survival and chemotaxis of BAFF-stimulated CLL cells. By contrast, targeting BTK or PI3K was less effective. All BCRi’s fully blocked canonical NFκB activation in BAFF-stimulated CLL cells (suggesting its dependence on BCR signaling), but none inhibited the non-canonical path- way. By contrast, pevonedistat, an inhibitor of Nedd8-activating enzyme which we have previously shown to abrogate TNFR-mediated NFκB activation, blocked both canonical and non-canonical NFκB activity in BAFF-stimulated CLL cells. SYK inhibitor entospletinib, but not other BCRi’s, decreased Mcl-1 expression in CLL cells co-cultured with BAFF-expressing stroma and abrogated BAFF-mediated upregulation of pSTAT3, a transcription factor which regulates Mcl-1. This was accompanied by a decrease in Mcl-1 transcript. BAFF receptor signals via the TRAF complex to induce non-canonical NFκB activation in neoplastic B-cells. We supposed that TRAF complex could be directly responsible for SYK activation by BAFF. Indeed, IP experiments demonstrated that SYK directly complexed with TRAF2/3 in BAFF-stimulated neoplastic B-cells.

Summary/Conclusions: Thus, BAFF-mediated induction of BCR-associated kinases and Mcl-1 contributes to CLL cell survival. SYK inhibition is a promising therapeutic strategy uniquely poised to antagonize crosstalk between BAFF and BCR, thereby disrupting the pro-survival microenvironment signaling in CLL.
S119
LOW MYBL2 EXPRESSION OBSERVED IN MYELODYSPLASTIC SYNDROME PATIENTS WITH WORSE PROGNOSIS IS ASSOCIATED WITH ALTERED DNA REPAIR MECHANISMS IN HAEMATOPOETIC STEM CELLS
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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.

Aim: 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 (5 Gy) and chemotherapy (CDDP) replication, apoptosis and colony formation 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 Mybl2+/– HSCs are less sensitive to inhibition of DNA-PKC (required for NHEJ) but not ATM (required by HR). We also observed that after ionizing irradiation Mybl2+/– 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 novel role for MYBL2 in DNA 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.

S120
A NOVEL GENETIC AND MORPHOLOGIC PHENOTYPE OF ARID2-MEDIATED MYELODYSPLASTIC SYNDROMES
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Background: Clinical heterogeneity of myelodysplastic syndromes (MDS) and related myeloid neoplasms reflects molecular diversity. Most common genetic associations with distinct clinical or pathomorphologic phenotypes have been described, but many other genetic somatic lesions exist and their clinical context still remains elusive. At rich interactive domain 2 (ARID2), which is located on chromosome 12q, encodes a component of the SWI/SNF complex that is involved in chromatin remodeling. In recent years multiple groups detected ARID2 mutations in a variety of solid tumors.

Aim: Here, we present whole exome sequencing–guided identification of novel ARID2 mutations in myeloid neoplasms. Specifically, in addition to copy number analysis and deep targeted and exome sequencing, here we include RNA sequencing and splicing analyses of the roles of splicingosomas in MDS and other myeloid neoplasms.

Methods: Bone marrow aspirates or blood samples were collected from 1,473 patients with MDS (n=455), myelodysplastic/myeloproliferative neoplasms (MDS/MPN) (n=201), myeloproliferative neoplasms (MPN) (n=56), sAML (n=221), and primary acute myeloid leukemia (pAML) (n=540) at the Cleveland Clinic and The University of Tokyo; the registered data at The Cancer Genome Atlas were also included. Diagnoses were classified using World Health Organization criteria. Informed consent for sample collection was obtained according to a protocol approved by each Institutional Review Board in accordance with the Declaration of Helsinki.

Results: By comprehensive genetic investigation of these cases, we characterized here cases (10%) in which decreased expression of ARID2 mediated their clinical effects in MDS and other myeloid neoplasms via multiple kinds of genetic lesions. We showed that insufficient ARID2 expression mainly in MDS arose from ARID2 mutations, deletions, and missplicing due to U2AF1 mutations that yield deleterious transcript. Clonal architecture analyses showed that ARID2 mutations and deletions occurred as initial events of MDS or myelodysplastic/myeloproliferative neoplasms, and not during progression to acute myeloid leukemia. Morphologically, progressive maturation in myeloid and erythroid lineages and hypolobated megakaryocytes (indicated by arrow heads in Figure 1) were common in cases with ARID2 mutations and deletions, and were also found in cases with U2AF1 mutations. Functionally, we utilized in vitro knockdown models of ARID2 expression in hematopoietic cell lines and bone marrow mononuclear cells. Since no homogenous deletion or mutation of ARID2 was identified, we transduced shRNA in neo-plastic and healthy hematopoietic cells to obtain disease models with partial reduction of ARID2 expression. Two myeloid cell lines (HL60 and K562) in which ARID2 expression was knocked down showed significantly lower cell counts compared to those with normal ARID2 expression, compatible with more apoptotic cells in knockdown experiments. Flow cytometric analysis of the cell lines with reduced ARID2 expression revealed increased cell-surface maturation markers, CD11b and glucosephorin A (GPA), suggesting that reduced expression of ARID2 resulted in more differentiation in myeloid and erythroid lineages. Knockdown of ARID2 failed to reduce colony formation in bone marrow mononuclear cells. These results indicate that reduced ARID2 expression might induce more differentiation in myeloid/erythroid lineages and more apoptosis to reduce cell populations without reduction of proliferation capacity in hematopoietic progenitor cells. Finally, we examined morphological findings associated with knockdown ARID2 expression. Compared to control cells, K562 cells with reduced ARID2 expression formed more hypolobated megakaryocytes, which confirmed morphological findings seen in ARID2 and U2AF1 defects.

Figure 1.

Summary/Conclusions: ARID2 is a MDS-suppressor gene whose expression is attenuated by multiple mechanisms as it shapes the distinct morphological phenotype of a subset of myelodysplasia.

haematologica | 2017; 102(s2) | 11

Madrid, Spain, June 22 – 25, 2017
THE VALUE OF NGS PANEL SEQUENCING TO MOLECULARLY DEFINE MYELOID 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.

Methods: According to WHO 2016, 1143 patients were molecularly 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 sample quality, were classified as "possible" AML (n=29), MDS (n=211), MPN (n=5), 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 definite 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 ELN 2014) significance. More than 1 gene mutation was found in 166/229 patients (72%), including information of adverse impact (e.g. of 68 NPM1 positive patients, 17 had DNM3TA mutations and 20 FLRT3-ITD). Following NPM1, RUNX1 was the second most frequently mutated gene (46/225; 20%) and mutations were significantly more common in patients with ≥ 3 aberrant genes (30/107; 28%) than ≤ 2 (12/118; 10%). A similar pattern was found in s-AML and t-AML. In the cohort of "possible" AML (including MDS overlap), 45/48 (94%) patients had ≥ 1 hit. Most frequently mutated were ASXL1 (16/48; 33%), TET2 (12/44) and SRSF2 (19/48; 41%). 16% had all three mutated. This combination is also seen frequently in the three-way interaction in CMML (10/44; 23%). In MDS, 124/157 (79%) cases showed mutations, of which 108 had ≥ 1 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 ≥ 2 AML/MPN overlap (36/190; 19%), reactive morphologic changes (17/201; 8%) or even unclear morphology (19/116; 17%). Of these three subsets, five patients had only the TET2 mutation with <10% burden, which is observed in clonal hematopoiesis of indeterminate potential (CHIP), too. However, using panel sequencing in cases with possible MDS, unclear or reactive morphology revealed 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, DNM3TA, TET2 mutations with <10% burden).

Summary/Conclusions: WHO 2016 requires information on numerous genes for diagnosis, prognosis and therapeutic decisions. This challenges conventional approaches, which determine all in one panel. We demonstrate the feasibility in routine settings for a broad spectrum of myeloid malignancies and identify 1) relevant patterns and mutation interactions; 2) genetic aberrations supporting diagnosis for samples with borderline morphology or poor quality and 3) patient-specific clonality usefulness for follow-up.

IDENTIFICATION OF ABERRANTLY SPliced GENES AND DEREGULATED PATHWAYS/Gene ONTOLOGY THEMES IN MYELODYSPLASTIC SYNDROME PATIENTS WITH SPlicing FACTOR GENE MUTATIONS


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Background: Myelodysplastic syndromes (MDS) and related myeloid disorders (“myelodysplasia”) are a heterogeneous group of clonal hematopoietic disorders with a highly variable clinical outcome.

Aims: The purpose of this study was to establish a novel gene expression-based classification of myelodysplasia for better prognostication.
Summary/Conclusions:

The Class-II signature was shown to be more dramatically up-regulated during risk acute myeloid leukemia based on the expression of 17 genes related to erythroid lineage. 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] 7.3 [95% CI, 1.3–41], P <0.01) 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.

Results: Unsupervised clustering of gene expression data of bone marrow blasts 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 (t-test HR 7.2 [95% CI, 3.0–17], 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-II 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 leukemia 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.

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.

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 the PD-L1 and PD-L2 loci. Moreover, up-regulation of PD-L1/PD-L2 genes is invariably associated with leukemic transformation (HR 7.3 [95% CI, 1.3–41], P <0.01) 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.

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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-II 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 leukemia 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.
shown previously to increase FOXO1 nuclear localization and activity, have been linked to diminished survival in DLBCL patients uniformly treated with rituximab-based immunotherapy. Although the contribution of FOXO1 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 FOXO1, and that inhibitors of the downstream BCR signaling pathways down-regulate CD20 expression. Therefore, here we sought to determine whether FOXO1 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 FOXO1 protein in CD20 regulation. qRT-PCR and Dual Luciferase Assays was done to determine the influence of FOXO1 on CD20 transcription and its effect on tumor cells. We aimed to discern tumor cells that respond the best to drugs and to unravel the molecular interaction between FOXO1 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 according to the guidelines and were approved by the Ethics Committee.

**Results:** To determine the potential role of FOXO1 protein in CD20 regulation, we disrupted FOXO1 focus using the CRISPR/Cas9 genome editing technology in Raji cells. In vitro complement-dependent cytotoxicity assay we show that ablation of FOXO1 results in upregulation of CD20 levels and improved resistance to rituximab efficacy. To see whether FOXO1-dependent up-regulation of CD20 transcript was translated into tumoral phenotype, we used molecular interaction between FOXO1 and CD20 promoter and performed EMSA and ChIP experiments. For animal studies we used SCID Fox Chase mice model. We found that mice with 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 FOXO1 regulate the CD20 promoter activity. In different B-cell lymphoma cell lines MK-2206 and GDC-0068 significantly downregulated the levels of M54A1 transcript (encoding CD20). Finally, using both EMSA and ChIP assays we detected specific binding of FOXO1 to the M54A1 promoter to the extent comparable to other known FOXO1 target genes.

**Summary/Conclusions:** Collectively, our results indicate that FOXO1 is strong negative regulator of CD20 expression and add new insights into the mechanisms underlying the contribution of FOXO1 mutations to the resistance of B-NHLs to R-CHOP therapy. In light of current knowledge and our observations presented in this study, FOXO1 inhibition represents a novel strategy to increase the efficacy of anti-CD20 monoclonal antibodies.

**Acknowledgements:** Abstract supported by national grants: NCN, Poland, projects no: 2013/11/B/ZZ5/02240 (BP) and 2015/18/E/NN6/00792 (MW); MNiSW, Poland, project no: DI2014027344 (NM) and European Comission (Horizon 2020, project no: 692180-STREAM-H2O20-TWINN-2015, CSA action (JG).

S126

**ALPHA-KETOGLUTARATE EXPOSES METABOLIC VULNERABILITIES IN B-CELL LYMPHOMAS**

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**Background:** Metabolic rewiring is a cancer hallmark. These metabolic changes can be secondary to a broader oncogenic-driven deregulation (e.g., MYC), or they may be more specific and result from mutations in enzymes (e.g., IDH1 and IDH2) that directly control energy flux in the cell. Mutant IDH acquires a neomorphic activity and aberrantly generates high levels of D2-HG/α-KG with attending impaired dioxygenase function. Together, these data suggested no: 2013/11/B/NZ5/03240 (BP) and 2015/18/E/NZ6/00702 (MW); MNiSW, Poland, project no: DI2014027344 (NM)) and European Comission (Horizon 2020, project no: 692180-STREAM-H2O20-TWINN-2015, CSA action (JG).

**Figure 1.**

**Summary/Conclusions:** We showed that α-KG induces growth suppression/apoptosis in mature B cell tumors, in vitro and in vivo. We demonstrated that proximally α-KG exerts its tumor suppressive effects by inhibiting ATP synthase activity, the effects of α-KG on cellular ATP levels, the measurement of AMPK activity and of the mTORC1 kinase.

**Results:** The cell-permeable DM-KG induced a marked dose-dependent growth suppression in a panel of DLBCL cell lines that encompasses the molecular heterogeneity of this disease (ABC, n= 5, GCB, n=5 and OxPhos, n=4). In most instances, the growth inhibition exceeded 80% of vehicle control exposed cells and it could be detected as early as 24h and reached its peak at 72-96h post-exposure (Figure 1A). In all cell lines examined, induction of apoptosis accounted for most of the anti-lymphoma effects of DM-KG. There was no clear segregation between the DLBCL molecular subtype and DM-KG-induced growth inhibition (e.g., the ABC cell lines Ly10 and Ly3 were the most sensitive and most resistant, respectively, Figure 1A). Remarkably, we found that normal mature B cells (murine and human) were resistant to growth inhibition and apoptosis induced by DM-KG (Figure 1C). Contrary to that, exposing viable primary CLL, FL and DLBCL cells to DM-KG significantly induced apoptosis (p<0.01) in all tumors examined (n=17). In mouse models of lymphoma, we showed that also pharmacological inhibition of FOXO1 activity upregulated surface CD20 levels. Moreover, we demonstrated that FOXO1 regulates the CD20 promoter activity. In different B-cell lymphoma cell lines MK-2206 and GDC-0068 significantly downregulated the levels of M54A1 transcript (encoding CD20). Finally, using both EMSA and ChIP assays we detected specific binding of FOXO1 to the M54A1 promoter to the extent comparable to other known FOXO1 target genes.

**Summary/Conclusions:** Collectively, our results indicate that FOXO1 is strong negative regulator of CD20 expression and add new insights into the mechanisms underlying the contribution of FOXO1 mutations to the resistance of B-NHLs to R-CHOP therapy. In light of current knowledge and our observations presented in this study, FOXO1 inhibition represents a novel strategy to increase the efficacy of anti-CD20 monoclonal antibodies.

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S127

**DELETION OF THE F-BOX PROTEIN NIPA (NUCLEAR INTERACTION PARTNER OF ALK) IMPAIRS NPM-ALK DRIVEN TRANSFORMATION**

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Background: ALCL is a high grade lymphoma characterized by anaplastic morphology, expression of CD30 (K-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 kinase. NPM-ALK infected NIPA deficient mice are 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 softagar assays to evaluate their transformation ability. Moreover, NIPA was downregulated through targeted genetic approaches in Karpas299 and NPM-ALK infected BarF3 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 Thy1.2+lymphoma with a latency of 4-6 months, developing neoplastic T-cell infiltration of bone marrow and lymphatic organs. Lymphomas were analyzed regarding immunphenotype and clinical presentation.

Results: Primary Nipa-/-MEFs infected with NPM-ALK were plated in softagar showed significantly reduced colony formation potential upon NPM-ALK expression (38 vs 79 CFUs; p<0.001). These results were substantiated in human and murine cell lines, where significantly reduced proliferation ability was observed in NIPA downregulated NPM-ALK expressing BarF3 cells (74% of ctrl; p<0.001) as well as in Karpas299 cells infected with NIPA miR (66% of wt growth; p<0.01). Moreover, treatment with the ALK inhibitor TAE-684 gave evidence of possible synergistic effects of ALK inhibition and NIPA knockdown. Mice transplanted with Lck-CreTG12; Myelo-MSNAIE infected bone marrow cells showed significantly prolonged disease development and survival (143d vs 79d in wt). Morphologically, mice presented with enlarged thymi, splenomegaly, lymphadenopathy, and bone marrow infiltration. Immunphenotyping showed a pure T-cell phenotype in Nipa-/- lymphomas, thus resembling wildtype. In a long-latency model of NPM-ALK expression in enriched HSCs, a significantly prolonged survival (110 vs 80 days; p<0.01) and reduction of spleen colonies (10 vs 28 colonies/spleen; p<0.001) as well as in Karpas299 infected with NIPA miR and Nipa-/-MEFs infected with NIPA deficient NPM-ALK expressing cells, whereas cell cycle distribution seems not to be altered in knockout cells.

Summary/Conclusions: Taken together, we were able to show that NIPA is crucial for cell proliferation and transformation upon NPM-ALK expression. Investigations of the NIPA knockout mouse in a clinical relevant ALCL model highlight the importance of the NIPA/NPM-ALK axis in lymphoma development. Further analyses may thus elucidate NIPA as a novel molecular target for therapeutic intervention.

S128 THERAPEUTIC EFFECTS OF CAN-NP-ALK: PRECLINICAL EXPERIENCE WITH A NOVEL ALK INHIBITOR FOR TREATMENT OF ALCL

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Background: Gene therapy for transfusion dependent beta-thalassemia, as an alternative cure to autologous 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 lenograstim 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 number 2014-004860-39).

Methods: Based on the efficacy and safety of the clinical 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-/β+) have been treated with GLOBE-transduced CD34+ cells at a dose of 16x10^6-19.5x10^6 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.

S129 LUSPATERCEPT INCREASES HEMOGLOBIN AND DECREASES TRANSFUSION BURDEN IN ADULTS WITH β-THALASSEMIA


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Background: Luspatercept (ACE-536), a fusion protein containing a modified activin receptor type IIB, is being developed for the treatment of β-thalassemia. Luspatercept binds to select TGF-β superfamily ligands (such as GDF11) reducing aberrant Smad2/3 signaling and promoting late-stage erythroid differentiation and increased hemoglobin (Hgb). Luspatercept corrected the effects of ineffective erythropoiesis in a mouse model of thalassemia (Surargani R, Blood, 2015). Increased Hgb has been tolerated in a phase 1 study in healthy volunteers (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 effects of luspatercept in patients (pts) with either transfusion-dependent (TD) or non-transfusion-dependent (NTD) β-thalassemia with key endpoints of erythroid response (including Hgb increase) and pt-reported quality-of-life (QoL) in NTD patients, and reductions in RBC transfusion burden in TD patients.

Methods: Inclusion criteria: age ≥18 yr and either TD (≥4 RBC U/8 weeks prior
to first dose, confirmed over 6 months) or NTD (<4 RBC U/I8 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 completed NCT01745940; ext ongoing NCT02268409).

Results: Sixty-three patients with TM and osteoporosis participated in the phase 2b clinical trial. Main inclusion criteria included adult patients (>30 years of age) with TM and BMD T-score between -2.5 and -4.0 in at least one of the evaluated 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 vitamin D; 12 months). 12% of patients were diagnosed with TM and 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.

**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 vitamin D; 12 months). 12% of patients were diagnosed with TM and 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 THALASSEMIA: THE FRENCH EXPERIENCE**

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### Background:

In clinical practice, allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment offering a definitive cure for patients with beta-thalassemia. Its outcome has improved over the last 3 decades with current 5-year disease-free survival up to 90% when transplants are 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 HSCT 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 HSCT. Study was based on data collected in the national registry of patients with beta-thalassemia and conducted in collaboration with the French DIGHO network. The outcome was correlated with the data 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).

### Results:

A total of 134 patients had received allogeneic HSCT for beta-thalassemia in France from 1985 to 2012. 107/134 patients experienced successful HSCT (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 infections (6 months). 125 patients were analyzed for long-term effects. Median age at HSCT was 5.9 years (8 month-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, similar to those observed in patients treated with transfusion and chelation therapy occurred after transplant in 12% of patients: 7 hypothryoidism, 2 heart failure, 5 diabetes. 2 patients had chronic respiratory failure related to transplant. The height SDS improved after HSCT if performed at a young age. Weight
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 (cRBC), 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 x 10^12 erythrocytes), and for iPSC we need virus- and transgene-free reprogramming protocols. To solve the above mentioned issues a customised humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1-10^12 erythropoiesis from PBMCs to pure adult EBL cultures within 25 days, comparable to non-GMP commercial media. To generate iPSC, a non-integrative polyclonostic episomal vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was reprogrammed with non-GMP commercial media. To generate iPSC, a non-integrative polyclonostic episomal vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was reprogrammed with non-GMP commercial media.

Summary/Conclusions: Here 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.

AML Biology I: Towards molecular therapies

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 several distinct MLL fusion proteins (MLL-FPs) were identified, it is not clear if transforming mechanisms are conserved across the entire family of MLL fusions.

Aims: We hypothesized that common oncogenic mechanisms are encoded in stable physical and genetic MLL-fusion-specific interaction networks. Thus, we aimed to identify common critical effectors of different MLL fusion proteins that are presumed to employ different mechanisms of oncogenic transformation.

Methods: Protein complexes of 7 molecularly distinct, affinity-tagged MLL-FPs (MLL-AF4, MLL-AF9, MLL-ENL, MLL-CBP, MLL-EE, MLL-GAS7 and MLL-AF1p) were purified from stable cell lines allowing for inducible, single-copy transgene 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 proteome 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 transformed mouse fetal liver cells resulted in loss of serial re-plating capacity in vitro and prolonged disease onset in vivo. Furthermore, knockdown of SETD2 caused a proliferative disadvantage in primary cells from AML patients with different MLL-rearrangements without affecting MLL-wild-type AML cells. We found that SETD2 was essential for efficient repair of DNA breaks, as SETD2-deficient leukemia cells showed increased levels of DNA damage and activation of p53, leading to the accumulation of mutations.

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 genomic complexity.
C/EBPα can act as a gain-of-function allele with distinct molecular properties. However, the molecular basis of C/EBPα p30-induced leukemogenesis is incompletely understood.

Aims: We hypothesized that the interaction between the oncogenic C/EBPα 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 CEBPA mutant AML to perturbation of 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 and colony formation. 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 C/EBPα and MLL co-localize on the C/EBPA and CEBPB promoters, indicating functional co-occupancy in gene regulation. To investigate the importance of different, annotated functional domains within the MLL protein in the context of C/EBPα p30 expression, we introduced targeted mutations across the MLL gene in Cebpap30/p30 cells using the CRISPR/Cas9 system. This analysis revealed a strong dependence of C/EBPα expression on the expression of an intact MLL protein. Surprisingly, loss of the enzymatic activity of Mll by mutagenic targeting of the SET domain did not significantly affect cell survival. In contrast, cells were particularly sensitive to mutagenesis of the Menin-binding motif in Mll. Mll targeting strongly induced myeloid differentiation in Cebpap30/p30 cells as measured by increased levels of cell surface markers. To further investigate the functional role of Mll 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 of MI-463 treatment indicated gene expression changes associated with myeloid differentiation, which could be confirmed by flow cytometry. Importantly, expression of C/EBPα 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 hypothesize that C/EBPα p30 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 C/EBPα 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 LEUKEMIA

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Background: Functionally critical decreases in PU.1 levels or activity are present across various different genetic and epigenetic subtypes of AML, and overall represent more than 50% of AML cases (Sive et al. Leukemia. 2016). However, approaches for the specific therapeutic targeting of these patients are thus far lacking.

Aims: Retrospective analysis of PU.1 expression has previously been exploited but efforts to pharmacologically target the PU.1 protein have been unsuccessful. Here, we tested the therapeutic potential of PU.1 binding to chromatin critically depends on additional minor groove contacts upstream of the core ETS binding motif, which determine selectivity for PU.1. We used an integrated screening strategy utilizing biosensor surface plasmon resonance, DNA footprinting, and cell-based dual-color PU.1 reporter assays. Finally, we explored additional cellular outcome tests as first-in-class PU.1 inhibitors. Targeted occupancy by our compounds in the minor groove induces perturbations in DNA conformation that are transmitted to the PU.1 site in the major groove and thus inhibit PU.1 binding via an allosteric mechanism. Functionally, treatment with 3 different compounds increased cell growth and colony forming capacity, increased apoptosis, and disrupted serial replating capacity of PU.1(p30) AML cell lines, and a majority of primary AML cell samples. ChIP and expression analysis showed that the compounds disrupt PU.1-promoter interaction and lead to downregulation of canonical PU.1 transcriptional targets in AML cells, confirming on-target activity.

Methods: We used tandem mass spectrometry to identify significant changes in transcriptional targets of PU.1, and selectivity over other ETS family members. Comparison with published transcriptomic and PU.1 ChIP-seq data sets, as well as ARACNe analysis of the PU.1 regulon in primary AML cells, demonstrated that the inhibitors antagonized PU.1-regulated pathways at a genome-wide level. Moreover, motif enrichment analysis pointed to decreased production of mature granulo-monocytic cells, consistent with PU.1’s known role in this lineage. However, this effect was reversible upon drug removal, and serial replating capacity was not affected suggesting no significant effects on normal HSPC. Lastly, in vivo treatment with PU.1 inhibitors in mouse models demonstrated strong antiproliferative effects and sensitization of the PU.1 regulon to anti-myeloid agents, in a manner consistent with the in vitro transcriptional targets of PU.1, and selectivity over other ETS family members.

Summary/Conclusions: We describe for the first time a strategy inhibiting PU.1 in AML, establishing proof-of-concept for this approach. Furthermore, we report the development of first-in-class PU.1 inhibitors which interfere with PU.1-DNA interaction through an allosteric, minor groove-mediated mechanism. Our work shows that it is feasible to pharmacologically target PU.1, and raises intriguing possibilities for the potential targeting of other transcription factors through minor groove-directed approaches.

S136 METABOLIC ADAPTATIONS TO TARGETED THERAPY IN FLT3 MUTATED ACUTE MYELOID LEUKAEMIA

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Background: FLT3 tyrosine kinase (TK) activating mutations (FLT3-mut) are amongst the most frequent in AML and are associated with a poor outcome. FLT3-mut promote constitutive activation of survival/proliferation pathways and drive cellular transformation. Gene expression changes associated with FLT3 TK inhibitors (TKI) have been described in a number of studies, usually 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 might play a significant role although the exact mechanisms are still ill-defined.

Aims: We hypothesised that metabolic adaptations facilitate FLT3 TKI resistance and aimed to identify early metabolic changes in FLT3-mut AML, following TKI treatment, in an attempt to unravel 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-mut AML cells. Gene expression changes 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-mut compared to FLT3 wild-type (FLT3wt) patient samples at diagnosis. We then confirmed that both human and murine FLT3-mut cells display increased glycolytic and respiratory capacity compared to FLT3wt cells. Gene expression changes associated with FLT3 TKI treatment were measured in the same conditions by RNA sequencing. Changes in viability and reactive oxygen species (ROS) in various culture conditions were measured by FACCS. 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-mut compared to FLT3 wild-type (FLT3wt) patient samples at diagnosis. We then confirmed that both human and murine FLT3-mut cells display increased glycolytic and respiratory capacity compared to FLT3wt cells. Gene expression changes associated with FLT3 TKI treatment 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.
FLT3 TK activity may improve the eradication of FLT3mutAML cells. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK inhibition by respectively counteracting oxidative damage and sustaining macromolecule biosynthesis and cellular energetics. 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 specifically counteracting oxidative stress following FLT3 TK inhibition by respectively counteracting oxidative damage and sustaining macromolecule biosynthesis and cellular energetics.

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.

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 Spi1 (FGRS), and reintroduction of a proper inductive niche—adult mouse ECs were reprogrammed into HSCs (rEC-HSCs) with multi-lineage engrafment 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 engrafment 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.

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 tress. ultraviolet radiation, ischemia/reperfusion (I/R) /tetal . However, it is still unclear if bone marrow mesenchymal stem cells (BM/MSC) 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 BM/MSC can rescue suffering endothelial cells by transferring mitochondria to endothelial cells through nanotubes.

Aims: To investigate the novel intercellular communication TNT between BM/MSC and BMdECs or HUVEC, illuminating its constituent and investigating the significance of transport of mitochondrial through TNT between BM/MSC 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 (BM/MSCs) and bone marrow-derived endothelial cells (BMdECs) or Human umbilical cord vein endothelial cells (HUVECs) respectively.

Results: Firstly, We observed the TNTs formed between BM/MSCs and endothelial cells including the TNT struc- ture between BM/MSCs and HUVECs or BMdECs are composed with F-actin, microtubule in addition to membrane. Live cell imaging showed the two xeno-geneic 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-INDUCED QUIESCENCE OF HEMATOPOIETIC STEM CELLS IN C57BL/6J MOUSE BONE MARROW

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Background: Some studies show that a high-fat diet (HFD) induces major perturbations in murine hematopoietic stem cells (HSC) and hematopoietic system homeostasis. 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 stress, 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 dormant HSC in vivo.

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 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-/anti-inflammatory cytokine levels were detected in blood and BM, respectively. The impact was observed after 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 dormant HSC in vivo.

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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-/anti-inflammatory cytokine levels were detected in blood and BM, respectively. The impact was observed after 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 dormant HSC in vivo.

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**Gene therapy, immunotherapy and vaccination 1**

**S141**

**WILMS' TUMOR 1 RNA- ELECTRO- PORATED 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 chemotherapy, but at very high risk of relapse with autologous DCs loaded with the human Wilms' tumor 1 (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 intra- dermally. WT1 mRNA levels in blood and marrow were followed as a measure of minimal residual disease. Circulating WT1-specific CD8+ T-cells obtained before vaccination and at the 4th dose were stained with anti-WT1 antibody and anti-HLA-A*0201 tetramers. To 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, harvested, and tested for 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 SEEER 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=0.001). In patients ≤65 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 5 who relapsed after DC vaccination; 2 proceeded to allogeneic stem cell transplantation, while the other 3 patients were brought back into CR by chemotherapy alone, 2 of them surviving more than 7 and 4 years respectively after reaching CR. Long-term clinical response was correlated with increased circulating frequencies of poly-epitope WT1-specific tetramer+CD8+ T-cells. Long-term overall survival was correlated with interferon-γ and tumor necrosis factor-α WT1-specific responses in DTH-infiltrating CD8+ T-lymphocytes.

**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 pursuing 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 patients with MM whose tumors express BCMA.

**Methods:** CRB-401 (NCT02656929) 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 (IMiD). In total, 10 patients entered a single dose study and 38 entered a phase 1 dose escalation trial (ORR) in the 9 evaluable patients is 100%, including 2 sCRs and 2 MRD-neg- ative responses (sCR and VGPR). CAR+ T cell expansion has been demonstrated consistently. An additional 6 months of follow up on reported results and initial data from an additional ~10 patients will be presented.

**Summary/Conclusions:** A total of 2 sCRs and ongoing clinical responses at 6 months with mild and manageable CRS to date. These initial data support the potential of CAR T therapy with bb2121 as a new treatment paradigm in MM.
Background: Fanconi anemia (FA), is a monogenic inherited syndrome associated with bone marrow failure (BMF), that has been considered a candidate disorder for hematopoietic stem cell (HSC) gene therapy. Up to date, three clinical trials have been performed, all of which failed to demonstrate engraftment of 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 ≥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. On average, 5 million CD34+cells/Kg were collected, with 45% recovery after immunoselection. The most relevant AE has been the transfusion of packed red blood cells and platelets. Six FA patients aged ≥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. The most relevant AE has been the transfusion of packed red blood cells and platelets. Six FA patients aged ≥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. The most relevant AE has been the transfusion of packed red blood cells and platelets. Six FA patients aged ≥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.

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 ANTI-GEN RECEPTOR T CELLS CONFFERS POTENT 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-like tyrosine kinase 3 (FLT3) 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-/luminescence-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 5x105 CAR-modified or control T cells (CD2: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 cytolytic activity 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+AML 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 allogeneic 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.
Background: Allogeneic hematopoietic stem cell transplant (HSCT) offers curative therapy for children who lack an available HLA-identical donor with hematopoietic disorders such as Primary Immune Disorders (PIDs), hemoglobinopathies, erythroid disorders and acute leukemias. CD3ε 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 CD3ε 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 (AP1903) dimerizes the Cas9 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 CD3ε 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>
<thead>
<tr>
<th>Non-Malignant</th>
<th>N=66</th>
<th>Malignant</th>
<th>N=38</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID</td>
<td>11</td>
<td>ALL (CR1 CR2 CR3)</td>
<td>24</td>
</tr>
<tr>
<td>WAS</td>
<td>4</td>
<td>AMI</td>
<td>14</td>
</tr>
<tr>
<td>CDG</td>
<td>3</td>
<td>CD4</td>
<td>4</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1</td>
<td>CD8</td>
<td>2</td>
</tr>
<tr>
<td>Sickle Cell Disease</td>
<td>5</td>
<td>B cells</td>
<td>2</td>
</tr>
<tr>
<td>Fanconi Anemia</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLI</td>
<td>5</td>
<td>Others</td>
<td>20</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, 2 with Grade 3 and one Grade 4 skin. Mild cGvHD was seen in 4 patients with Grade 2 GvHD with rapid resolution of symptoms, as it did in 2 patients, moderate cGvHD in 2 patients and one case of severe cGvHD in a patient with Grade 4 skin. HbGδd was seen in 2 patients, moderate cGvHD in 2 patients and one case of severe cGvHD in a patient with Grade 4 skin.

Summary/Conclusions: This data suggest that infusion of BPX-501 modified 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.
for a selected guide RNA confirmed no detectable genomic cleavage at over 75 mC/mG and statistical analysis revealed 100% efficiency and high quality of guide RNA. These experiments support the further development of specific CRISPR-Cas9 editing strategies of HSPOs to treat SCD and β-Thal patients.

S148
EXPOSURE TO INFECTION TRIGGERS Pax5 and ETV6-RUNX1 CHILDHOOD BCP-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 BCP-ALL subtype can be triggered by 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+/−-infected model (1), which were exposed to a common infection environment. These represent childhood BCR-ABL1p190 BCP-ALL and the most common subtype ETV6-RUNX1 BCP-ALL. Both model systems enable Sca-1-directed expression of BCR-ABL1 p190 or ETV6-RUNX1 in HSC and non-HSC cells, which can be manipulated by infection and how the pre-leukemic clone evolves to BCP-ALL.

Results: In summary, exposure to common pathogens can increase 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 plug formation and clearance. The 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 cytokine 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 storage.

Methods: Analysis of Rhoa, Rac1 and Cdc42 activity was performed using GST-Rhotekin and GST-PAK effector domain pulldown assays. Platelets were obtained from anticoagulated (CPD or EDTA) human, Rhesus monkey and murine whole blood. G04, N53C2766 and Casin, specific inhibitors for Rhoa, Rac and Cdc42, respectively, were utilized and used at concentrations of 75 mM, 50 mM and 10 mM, respectively. Rhoa deficient murine platelets were obtained from poyt1-C treated Mx1-Cre/Rhoa−/− 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/monkey platelets were stored at room temperature (RT) or 4°C (367/368/369) 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 7-14-day storage restores Rhoa activity to normal levels and allows normal extent of shape change and spreading on fibronectin. To analyze the kinetics and hemoimmunophenotypic properties of cold treated human platelets after xenotransfusion, we analyzed the survival of xenotransfused human platelets after long-term cold (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 transplanted 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 congeneric or autologous transfusion in all aspirinated mice and 80% of aspirinated Rhesus monkeys, respectively. Rhoa inhibition blocks the process of intracellular trafficking of GP through lipid rafts and endocytotic intermediates as assessed by confocal microscopy of GpIIb and the vacuolar sorting protein VPS33b, as well as biochemical fractionation of detergent-insoluble membrane lipid rafts, RhoA and G04, and 80% of aspirinated Rhesus monkeys, respectively.

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 LYMHPHOMA AND NEGATIVE INTERIM PET: FINAL RESULTS FROM PLATINUM BASED RANDOMIZED PHASE 3 TRIAL HD18 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 sublineages are present that are likely the result of clonal branched evolution. Understanding this clonal evolution and the order at 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− hematopoietic stem/progenitor cells (HSPCs) from the same samples were also isolated 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. Every 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 independent leukemic clonal initiation events. Instead, a more stepwise 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 new target in RPL10 R98S defective T-ALL.

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 due to a metabolic contribution in pediatric T-ALL remains unclear. Treatment intensification and risk stratification has reduced the relapse rate of T-ALL to ~15% but further improvements will require strategies that focus on specific subtypes as RPL10 R98S; if the long-term sequelae of toxic therapy are to be avoided.

Aims: 1) Explore the oncogenic contribution of the RPL10 R98S mutation in pediatric 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 differences between RPL10 WT and R98S expressing Ba/F3 cells. Hits were confirmed by western blot in lineage negative (lin−) 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 DiHydorhodamine 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/IL2γ−/− (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 lin− 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, explaining 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% reduction 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 negative weights of healthy NSG mice. In contrast, 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 waste 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).
OXIDATIVE STRESS AS A TARGET FOR HIGH-RISK LEUKAEMIA IN a mutation in T-ALL that is linked to alterations in cellular serine biosynthesis.

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established oncogenic cascade in T-ALL. Moreover, this is the first description of transcription factor Ikzf2. Alterations were also found in the JAK-STAT signaling, an translational efficiency still needs to be validated at the protein level, and the translation and proliferation: the atypical MAP kinase Mapk6, whose reduced trans-

the RPL10 R98S mutation reveals alterations for genes involved T cell differen-

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expressing cells enhance their endogenous serine production, leaving more medium from RPL10 R98S Ba/F3 cells contained higher residual serine levels cell cultures derived from Rpl10 R98S knock-in mice. Interestingly, exhausted translational efficiency of the encoding gene. Elevated Psph protein levels were (Psph), a key enzyme in serine biosynthesis. Ribosome footprinting revealed showing 5-fold upregulated protein levels was phosphoserine phosphatase Lif, Socs1, Pim1, Osm, Il10ra, Cish and Il21r. Another interesting candidate especially, the results from the mass spectrometry and the polysomal RNA sequencing datasets showed a significant enrichment and upregulation of members of the JAK-STAT signaling pathway with Casf2/z2, Jak1 and several Stats being 1.3-fold elevated at the protein level and higher translation efficiency for Lf, Ctn, Il10ra, Cish and Il21r. Another interesting candidate

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

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: 3704 approved drugs and pharmacologically active compounds were initially screened against an infant ALL cell line with MLL-rearrangement, PER- 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 11 standard childhood leukaemia cell lines and seven solid tumour 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. Nr2f2 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 previously 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 cell cultured translation through induction of 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-

Summary/Conclusions: In summary, we 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 leukaemia.

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 R98 mutated residue contacts the catalytic core (phosphotransferase center, PTC) of the ribosome and causes ribosome biogenesis, Ptd rib, chl, that defines targets for Met and Amino acid cells. These observations 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 translational and potentially driving oncogenicity.

Methods: We performed ribosome footprinting (RNA sequencing of ribosome bound RNA), polyosomal 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, polysonal 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, Mapk8 presented reduced translational efficiency in the ribosome footprinting, potentially due to differences in ribosome occupancy of an upstream ORF, whereas the transcription factor Ikrz2, a master regulator of the upregulated transcripts, was overexpressed at the transcriptional and protein level. Interest-

P154

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

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Polychemotherapy resistance is a major challenge in the treat-

27
tion. IC50 measurements showed that TP53 mutations lead to resistance against current TKIs and alkylating agents, but not against other drugs. The upstream pathway of p53 (CHK1, CHK2) and DNA damage recognition (γH2AX) were not impaired in the six ALL cell lines. To study the effect of TP53 mutation on resistance to treatment in more detail, we focused on the R248P mutation, located in hot spot codon 248, that we found in a relapsed patient with non-response to treatment and in the NHH cell line. Using a CRISPR/Cas9 knockout (KO) of endogenous p53 and lentiviral based re-expression in NALM-6, we generated p53 KO, and KO+w1 p53, KO+R248P and KO+GFP cell lines. The KO cells showed a similar resistance to DNA damage inducing drugs as KO+R248P cells. Overexpression of wt p53 in KO cells did not improve sensitivity to DNA damage inducing drugs. In contrast to wt p53, R248P did not inhibit cell proliferation under drug treatment. We found that this mutant was unable to induce downstream targets of p53 (p21, BAX). Moreover, ChiP-seq showed that R248P cannot bind the promoter and induce expression of typical p53 targets MDM2, p21, BAX, BCC3/PUMA, FA15 and P53R2. This result indicates that R248P is defined by loss of the consensus element of p53. However, the binding motif analysis showed that the R248P mutant still binds DNA at a different and purine-rich sequence. In summary, R248P leads to loss of wt p53 function and mediates resistance to topoisoasemase II inhibitors and alkylating agents.

Summary/Conclusions: Overall, our results show that mutations affecting TP53 hot spots, in particular codon 248, are associated with resistance of ALL cells to chemotherapy and reveal first insights into underlying mechanisms and pathways.
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/FLRT3/UK inhibitor fostamatinib. However, SYK activation 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, ento 0.03%, or ento 0.07% chow. Cohorts of mice were sacrificed 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 without alterations in total SYK protein levels. In general, PD inhibition of SYK target phosphoproteins was more pronounced in 0.07% ento-treated animals.

Summary/Conclusions: Constitutive activation of SYK signaling occurs frequently in childhood Ph-like and infant KMT2A-R childhood B-ALL. Ento treatment of B-ALL PDX models potently inhibited SYK pathway signaling proteins and significantly inhibited leukemia proliferation in vivo.

P159

PHARMACOLOGICAL ACTIVITY OF CB-103 – AN ORAL PAN-NOTCH INHIBITOR WITH A NOVEL MODE OF ACTION

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Background: NOTCH signalling is a developmental pathway known to play critical roles during embryonic development as well as for the regulation of self-renewing tissues. Aberrant activation of NOTCH signalling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmarks of cancer. When the NOTCH pathway is inappropriately activated by genetic lesions (over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations), it becomes a major driver for NOTCH-dependent cancers and resistance to standard of care treatment. Over 250’000 patients are annually diagnosed with NOTCH dependent cancers, with no specific therapy available to date.

Aims: Given the importance of NOTCH signalling in human cancers, several therapeutic approaches have been utilized to block NOTCH signalling. Two of these strategies are: a) the use of monoclonal blocking antibodies (mAbs) against NOTCH ligands and receptors and b) the use of small molecule gamma-secretase inhibitors (GSIs). However, these approaches can only be effective if tumor cells express full-length ligand or receptor molecules. As validation of NOTCH as a therapeutic target, clinical activity of these in clinical studies 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

P160

IKZF1 Δ4-7 can be easily screened by PCR but does not predict outcome in adults with acute lymphoblastic leukaemia: data from 490 patients enrolled on the UKALL14 trial


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Background: The IKZF1 gene encodes the IKAROS zinc-finger transcription factor and master regulator of lymphocyte differentiation. IKZF1 lesions are common in acute lymphoblastic leukaemia (ALL) and have been reported as independent prognostic factors for poor outcome. IKZF1 Δ4-7, resulting in the dominant negative IK6 isoform is the most common single IKZF1 deletion.

Aims: We aimed to generate and validate a simple, PCR-based screening assay for IKZF1 Δ4-7 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 IK6 expressing cell line SUP-B15 and calculated to be 0.001%. A total of 65 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 IKZF1 Δ4-7 was detected in 97/490 (20%) patients but the frequency varied by genetic subtype. Patients with BCR-ABL1 fusion had the highest IKZF1 Δ4-7 frequency (46/150, 31%) followed by patients with B-other ALL (29/154, 19%). Patients with other classic cytogenetic aberrations harboured significantly fewer IKZF1 Δ4-7 - low hypodiploidy (3/26), MLL gene fusions (3/31), t(1;19) and iAMP21 (0/3). MLPA did not detect any IKZF1 Δ4-7 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 IKZF1 Δ4-7 deletions undetected by MLPA, consistent with the higher sensitivity of this approach. Interestingly, three of these samples harboured alternative IKZF1 deletions in addition to IKZF1 Δ4-7. 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 main 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 IKZF1 Δ4-7 on achievement of CR, persistence of minimal residual disease (MRD) at x > 1 x 10^(-4) (lgT/CR quantitation by EuroMRD criteria) after courses 1 and 2 of therapy, EFS, OS and time to relapse, at a median follow-up of 23.1 months. Two thirds of patients (44/66) with IKZF1 Δ4-7 were MRD positive at the end of phase 1 compared with 147/273 (54%) patients without the deletion (p=0.059). However, this relationship between IKZF1 Δ4-7 and MRD did not persist after phase 2. We did not identify any association between IKZF1 Δ4-7 and any of the other outcome parameters tested.

Table 1.

Summary/Conclusions: IKZF1 Δ4-7 can be detected by a simple and cheap PCR assay, which is more sensitive than MLPA. The frequency of IKZF1 Δ4-7 was broadly comparable with previous studies. However, we did not find an association between IKZF1 Δ4-7 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 residual disease (MRD) monitoring in acute lymphoblastic leukaemia (ALL) cases, except of Ph-positive ALL. However in infant ALL, where MLL gene rearrangements are found the majority of cases, MLL FGTs are attractive targets for MRD detection.

Aims: To estimate prognostic significance of MRD 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 0.03-11.83) were included in the current study. Among them there were 39 (51.3%) MLL-AF4-positive cases, 14 (18.4%) MLL-MLLT1-positive, 12 (15.8%) MLL-MLLT3-positive, 6 (7.9%) MLL-MLLT10-positive, 4 (5.3%) MLL-EPS15-positive cases. MRD detection was performed in BM samples by real-time quantitative PCR and nested RT-PCR with sensitivity non-less than 10^-4. 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 were obtained in all cases.

Results: We confirmed our earlier finding that the most informative TP for the MLL-baby protocol was TP4. MLL-MLLT1-positive patients stratified to high-risk arm of MLL-baby protocol (EFS 0.05±0.04 vs 0.78±0.07 < p<0.0001; cumulative incidence of relapse 0.78±0.10 vs 0.11±0.07 < p<0.0001, respectively) and for all others MLL-rearranged patients treated by intermediate risk (imR) arm (EFS 0.00 vs 0.71±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 MRD-negative patients. Multivariate analysis revealed that initial CNS disease (hazard ratio (HR) 2.703, 95% CI 1.255-5.284, p=0.011) and MRD positivity at TP4 was an independent factor of unfavorable outcome in infants with MLL-rearranged ALL enrolled into MLL-Baby protocol irrespective of treatment arm. Treatment intensification for MRL-positive at TP4 ImR patients did not improve their outcome. MRD-positivity at TP4 in ImR group was associated with MRD-positivity by flow cytometry on day 15, MLL breakpoint positions within intron 11 gene and initial CNS disease.

Summary/Conclusions: MRL monitoring by detection of MLL FGTs was feasible and had significant prognostic impact. MRD-positivity at TP4 was an independent factor of unfavorable outcome in infants with MLL-rearranged ALL enrolled into MLL-Baby protocol irrespective of treatment arm. Treatment intensification for MRL-positive at TP4 ImR patients did not improve their outcome. MRD-positivity at TP4 in ImR group was associated with MRD-positivity 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 ETV6-RUNX1 (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/ALL 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 (CD19+/CD2+/CD7-), null (CD1+/CD2+/CD7+), null (CD1+/CD2+/CD7+), neoplastic/mature (sCD3+). Recognition of pan-T cell CD antigen (pTag) expression included: CD1a, CD2, sCD3, CD4, CD5, CD7, CD8. ETP-ALL/lmimmunophenotype was defined as follows: absent (up to 5% positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD5 expression, 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 (LBP): 27%, age 35%; 72s, males: 67%; mediastinal mass (MM): 92%, primary CNS+: 8%. Immunophenotype: pro-T: 21% (pre-T: 17%, cortical or CD1a+, medullary/mature: pre-B: 3/9(33%) are alive, 3/4(75%) pts with pre-T and only 1/9((11%) with pro-T. 55% (0.418, 0.685) for ETP and non-ETP pts, respectively. Among ETP pts, (0.072, 0.272). There was no significant difference in OS and DFS in pts with 53% (0.152, 0.494) and 27% (0.097, 0.452) for pts without CD2, CD1a and 3 or less pTag present was 64% (0.511, 0.776), 66% (0.512, 0.803) and 64% (0.5, 0.782) compared to 11% (0.034, 0.256), 32% (0.152, 0.494) and 27% (0.097, 0.452) for pts without CD2, CD1a and pTag expression, absent or dim (75% positive cells) CD5 expression, expression: pro-T (CD2-), pre-T (CD2+), cortical (CD1a+), medullary/mature (sCD3+). Recognition of pan-T cell CD antigen (pTag) expression included: CD1a, CD2, sCD3, CD4, CD5, CD7, CD8. ETP-ALL/lmimmunophenotype was defined as follows: absent (up to 5% positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD5 expression, expression (25% positive cells) of 1 or more myeloid (CD13,CD33, CD15) or stem cell (CD34, HLA-DR) markers.

Methods: Induction chemotherapy for patients in the Northern Italy Leukemia Group 10/07 trial (ClinicalTrials.gov NCT-00795756; Blood 2016;128:176-182). Between 2007 and 2010 71 pts with Philadelphia-negative ALL were treated according to the GMALL 05/93 and T-LBL/2004 protocols. Immunophenotype was determined by immunohistochemical staining and by FCM of tissue aspirates if peripheral blood and bone marrow were uninvolved. We also evaluated prognostic value of immunophenotype according to WHO 2008 subtype and ETP6-RUNX1 (Early-T Cell Phenotype) definition in adult patients with T-LBL/ALL treated on uniform ALL protocol.

Results: 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 ETV6-RUNX1 (Early-T Cell Phenotype) definition in adult patients with T-LBL/ALL treated on uniform ALL protocol.

Figure 1. 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-T/pre-T-cell origin. ETP phenotype was non-significant factor for OS/DFS (p=0.10, 0.19) unless consistent with pro-T phenotype (CD2-), only 1/9 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:** UKALL60+ 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 patient choice; Arm A= Philadelphia chromosome positive (Ph+), Arm B= Non-intensive, Arm C= Intensive, Arm D= Intensive+, and Arm E= Registration only (in which treatment is at patient choice). Aims include determining appropriate regimens for future studies of novel agents in older people with ALL.

**Results:** Of 210 adult ALL pts, we identified 63 (30%) consecutive pts with elderly ALL. The average age at time of diagnosis was 67 (60–82), & 38 (60%) were males. Median follow up was 16.1 months (0.2–126), during which time 49 (63%) deaths occurred; 25 (63%) related to the disease, & 15 (37%) secondary to infection or other causes. Baseline characteristics at time of diagnosis: 54 (86%) pts had B-cell phenotype, 19 (35%) were Ph+. Only 9 (14%) pts had T-cell phenotype. 20 (31%) pts had a Charlson Comorbidity Index ≥2 & 17 (27%) presented with ECOG PS ≥2. Median Hgb was 10.6 g/dl (4.9–18.5), WBC 62 x 10⁹/l (0.5–160.5), PLT 51 x 10⁹/l (4–750), peripheral blast 30% (0–95), marrow blast 87.5% (0–100), & LDH 381.5 U/L (141–8440). Lymphoblastic lymphoma was only evident in 3 (5%) pts. Among pts with available data, 34/58 (59%) had B symptoms, 16/57 (28%) lymphadenopathy, 7/57 (12%) pleural effusions & 10/45 (22%) of pts had CNS leukemia. Cytogenetics at time of diagnosis: Of 48 pts with available data, 20 (41%) had complex cytogenetics (≥5 abnormalities), 18 (38%) had a monosomic karyotype, 8 (17%) were hypodiploid, 4 (8%) were hyperdiploid, & 2 (4%) were a mix of hypo- & hyper-diploid. FISH studies were available for 50 pts: 10 (20%) had CDKN2A del, 2 (4%) E2A-PBX1, 1 (2%) E2A-PBX1 [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 rate of 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.

**P165**

**CLINICAL OUTCOMES OF ELDERLY ACUTE LYMPHBLASTIC LEUKEMIA/LYMPHOMA – A SINGLE INSTITUTION EXPERIENCE**

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**Background:** Elderly acute lymphoblastic leukemia/lymphoma (ALL) is a rare disease with a poor prognosis and is underrepresented in clinical trials. This could be due to comorbidities, early death during induction, lower rates of complete remission, and higher risk of relapse with poor biological features (Gokbuget, Blood, 2013).

**Aims:** Describe clinical outcomes and prognostic factors of elderly ALL.

**Methods:** After IRB approval, we performed a retrospective study of patients (pts) aged ≥60 diagnosed with ALL from 2000 to 2016 at Mayo Clinic Rochester. Statistical analysis was performed using JMP 10.0 software.

**Results:** Out of 210 adult ALL pts, we identified 63 (30%) consecutive pts with elderly ALL. The average age at time of diagnosis was 67 (60–82), & 38 (60%) were males. Median follow up was 16.1 months (0.2–126), during which time 49 (63%) deaths occurred; 25 (63%) related to the disease, & 15 (37%) secondary to infection or other causes. Baseline characteristics at time of diagnosis: 54 (86%) pts had B-cell phenotype, 19 (35%) were Ph+. Only 9 (14%) pts had T-cell phenotype. 20 (31%) pts had a Charlson Comorbidity Index ≥2 & 17 (27%) presented with ECOG PS ≥2. Median Hgb was 10.6 g/dl (4.9–18.5), WBC 62 x 10⁹/l (0.5–160.5), PLT 51 x 10⁹/l (4–750), peripheral blast 30% (0–95), marrow blast 87.5% (0–100), & LDH 381.5 U/L (141–8440). Lymphoblastic lymphoma was only evident in 3 (5%) pts. Among pts with available data, 34/58 (59%) had B symptoms, 16/57 (28%) lymphadenopathy, 7/57 (12%) pleural effusions & 10/45 (22%) of pts had CNS leukemia. Cytogenetics at time of diagnosis: Of 48 pts with available data, 20 (41%) had complex cytogenetics (≥5 abnormalities), 18 (38%) had a monosomic karyotype, 8 (17%) were hypodiploid, 4 (8%) were hyperdiploid, & 2 (4%) were a mix of hypo- & hyper-diploid. FISH studies were available for 50 pts: 10 (20%) had CDKN2A del, 2 (4%) E2A-PBX1, 1 (2%) E2A-PBX1 [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 rate of 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.

**P166**

**MANAGEMENT AND OUTCOME OF ADULT PH+ ACUTE LYMPHBLASTIC LEUKEMIA PATIENTS TREATED AT THE “SAPIENZA” UNIVERSITY BETWEEN 1996 AND 2016**

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**Background:** All Ph+ve ALL patients (pts) aged ≥60 diagnosed with ALL from 2000 to 2016 at Mayo Clinic Rochester.
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 (18-88) 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).

Figure 1: The trend was also observed in patients of the intermediate-risk group but not in the standard-risk group. In univariate analysis, CD9 positivity (HR=2.4; p=0.022) and ETV6-RUNX1 translocation (higher prevalence in CD9+ patients; p=0.001) were significantly more common in the intermediate-risk group (P=0.044) and a higher proportion of CD9+ patients was stratified into the high-risk group (P=0.025). Besides, CD9- patients had poorer prednisone response (P=0.014). The 5-year overall survival (OS) and relapse-free survival (RFS) rates of CD9+ patients were significantly lower than those in CD9- patients (P=0.029). Subgroup analysis revealed remarkably poorer outcomes in CD9+ patients of the high-risk group (P=0.045). A similar trend was also observed in patients of the intermediate-risk group but not in the standard-risk group.

Summary/Conclusions: Our data indicate that expression of CD9 was significantly associated with inferior survival outcomes in pediatric B-ALL patients. The observation of patients with no risk factors for poor outcome, suggesting that CD9 expression could potentially be used in conjunction with other known prognostic factors for refinement of risk group stratification. Our study also lays the foundation for future development of CD9-targeted therapy for high-risk and relapsed/refractory pediatric B-ALL.

P167 THE TETRASPANIN CD9 IS A PROGNOSTIC MARKER FOR PREDICTING SURVIVAL OUTCOMES OF PEDIATRIC B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-precursor acute lymphoblastic leukemia (B-ALL) is the most common childhood malignancy, accounting for approximately 30% of pediatric cancers. With advances in risk-adapted chemotherapy, the overall cure rate of newly diagnosed B-ALL is approaching 85% in most developed countries. However, relapse still occurs in ~20% of patients and a significant portion of them are not initially classified in the high-risk disease entity, underlining the need for development of additional informative prognostic biomarkers.

Aims: CD9, a tetraspanin family protein, regulates multiple physiologic processes including cell migration and adhesion, and has been associated with metastasis and progression of various types of cancers. In this study, we aim to evaluate its expression pattern and prognostic significance in pediatric B-ALL.

Methods: Cell surface CD9 expression on leukemic blasts at disease presentation was characterized by multicolor flow cytometry in a cohort of pediatric B-ALL patients. The CD9 expression status was correlated with clinical parameters, including age, sex, white cell count, cyogenetics and prednisone response, Kaplan-Meier survival analysis was performed to investigate the possible association of CD9 expression with clinical outcomes. The potential role of CD9 expression as a predictor of 5-year survival outcomes was evaluated using Cox regression models.

Results: Among 118 cases tested, blasts of 92 patients (78.0%) were CD9+ (30% of CD9-expressing blasts). There were no significant differences in age, sex and white cell count between CD9+ and CD9- patients. Major cytogenetic subgroups were similarly distributed except for hyperdiploidy (all patients were CD9+; P=0.022) and ETV6-RUNX1 translocation (higher prevalence in CD9- patients; P=0.001). Significantly more CD9+ patients were stratified into the intermediate-risk group (P=0.044) and a higher proportion of CD9+ patients was stratified into the high-risk group (P=0.025). Besides, CD9- patients had poorer prednisone response (P=0.014). The 5-year overall survival (OS) and relapse-free survival (RFS) rates of CD9+ patients were significantly lower than those in CD9- patients (P=0.029). Subgroup analysis revealed remarkably poorer outcomes in CD9+ patients of the high-risk group (P=0.045). A similar trend was also observed in patients of the intermediate-risk group but not in the standard-risk group.

Summary/Conclusions: Our data indicate that expression of CD9 was significantly associated with inferior survival outcomes in pediatric B-ALL patients. The observation of patients with no risk factors for poor outcome, suggesting that CD9 expression could potentially be used in conjunction with other known prognostic factors for refinement of risk group stratification. Our study also lays the foundation for future development of CD9-targeted therapy for high-risk and relapsed/refractory pediatric B-ALL.

P168 PEDIATRIC MLL ACUTE LEUKEMIA PATIENTS SHOW DIFFERENTIAL HDAC EXPRESSION

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Background: Overexpression of histone deacetylases (HDACs) is a common feature in acute leukemias. Consequently, HDAC inhibitors (HDACi) have emerged as promising targeted therapy. However, non-specific HDACi may lead to accumulation of double stranded DNA lesions, so more selective isoform specific HDACi are needed. Expression patterns of HDACs in childhood leukemia have been scarcely studied.

Aims: To analyze the expression of HDAC isoforms in different subtypes of pediatric leukemia and correlate them with prognosis and clinico-biological features.

Methods: We evaluated the mRNA gene expression profile of class I, II and IV HDAC genes (HDAC 1-11) by quantitative PCR in 126 leukemic pediatric samples and a pool of non-neoplastic samples as calibrator. Patients were treated according to the Spanish Hemato-Oncology Cooperative Group protocols in a single center. The HDAC expression levels in different groups were compared by the Mann-Whitney test. The level of significance was set up at p<0.05. The analyses were performed with SPSS 24.0.

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 the benefit 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 0.7-17.4 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) received 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 68% for BCP-ALL, 52% for TALL and 67% for AML patients (p=0.0001). 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).

Summary/Conclusions: We have observed a specific pattern of HDACs expression in pediatric patients with MLL rearrangement. Our study, if further confirmed, suggests that specific HDACi would potentially be a useful targeted treatment for pediatric patients with MLL rearranged leukemia.

P169 MINIMAL DISSEMINATED DISEASE DETECTION BY FLOWCYTOMETRIC IMMUNOPHENOTYPING IN T-CELL ACUTE LYMPHOBLASTIC LYMPHOMA G.K. Viswanathan1,*, P. Tembhare1, N. Patkar1, S. Gujral1, P.G. Subramanian1

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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 in PB and BM using 8-10 colour flowcytometric immunophenotyping and evaluate the clinical and immunophenotypic features.

Methods: This study included retrospective analysis of 42 patients 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 leukocyte count and platelet counts. LDH was raised in majority of the patients (Mean 674±UL; N=190±UL). 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 morphology. 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).

Figure 1.

Summary/Conclusions: Our study shows that minimal disseminated disease is seen in more than one-fourth of cases (28.6%) of T-LBL with <25% blasts in PB and BM. This underlines the importance of flowcytometric evaluation of bone marrow in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as studies have shown inferior event free survival in T-LBL with minimal disseminated disease as compared to patients without minimal disseminated disease.

P170 INOTUZUMAB OZOGAMICIN IN COMBINATION WITH LOW-INTENSITY CHEMOTHERAPY (MINI-HYPER-CVD) AS FRONTLINE THERAPY FOR OLDER PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: UPDATED RESULTS FROM A PHASE III TRIAL N. Shurtleff1, H. Kantarjian1, S. Abruzzese1, L. Vose1, R. Callaghan1, E. Jabbour1, D. Thomas1, G. Garcia-Manero1, N. Daver1, G. Borthakur1, N. Jain1, M. Konopleva1, K. Sasaki1, N. Pennmaraju1, Y. Alvarado1, J. Jacob1, R. Garris1, P. Thompson1, J. Cortes1, E. Jabbour1

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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 (io), 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.

Figure 1.

Summary/Conclusions: Our study shows that minimal disseminated disease is seen in more than one-fourth of cases (28.6%) of T-LBL with <25% blasts in PB and BM. This underlines the importance of flowcytometric evaluation of bone marrow in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as studies have shown inferior event free survival in T-LBL with minimal disseminated disease as compared to patients without minimal disseminated disease.
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 (78%) 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 pts older 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).

Treatment was overall well-tolerated. The median times to platelet and ANC recovery in cycle 1 were 22 days (range, 11-91 days) and 16 days (range, 0-49 days), respectively. In cycles 2-8, the median times to platelet and ANC recovery were 22 days and 17 days, respectively. Prolonged thrombocytopenia (lasting >6 weeks) occurred in 37 pts (79%) at some point during therapy; 8 pts (17%) experienced prolonged thrombocytopenia during induction and 36 (77%) during 1 or more subsequent courses. Grade ≥3 transaminase elevation occurred in 9 pts (19%), hyperbilirubinemia in 8 (17%) and hemorrhage in 7 (15%). 4 pts (9%) developed VOD (1 after ASCT, 3 unrelated to ASCT). 8 pts (17%) experienced prolonged thrombocytopenia during induction and 36 (77%) during 1 or more subsequent courses. Grade ≥3 transaminase elevation occurred in 9 pts (19%), hyperbilirubinemia in 8 (17%) and hemorrhage in 7 (15%). 4 pts (9%) developed VOD (1 after ASCT, 3 unrelated to ASCT). 8 pts (17%) experienced prolonged thrombocytopenia during induction and 36 (77%) during 1 or more subsequent courses. Grade ≥3 transaminase elevation occurred in 9 pts (19%), hyperbilirubinemia in 8 (17%) and hemorrhage in 7 (15%). 4 pts (9%) developed VOD (1 after ASCT, 3 unrelated to ASCT).

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-PLKHO1 (three patients), MYB-PELKH1 (three patients), MYB-DCPS (one patient), and MYB-MIR2134 (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;16), who harbored MYB-PELKH1. 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-PELKH1 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 tumor–germline sequencing of paired tumor–germline 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 missense mutation in KMT2D 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, ZRS2, 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.

P172

BRANCHED CHAIN ACID METABOLISM REGULATES ALPHA-KETOGLUTARATE HOMEOSTASIS RESEMBLING MUTANT-IDH DRIVEN DNA HYPERMETHYLATION IN AML

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NPM1 in edited cells was confirmed by immunofluorescence. Return of NPM1 function of a reporter GFP-NPM1 fusion construct, and re-localization cytoplasmic NPM1wt allele was intact. The novel edited alleles could direct nuclear localization of mutant NPM1, we demonstrated that cytoplasmic localization of NPM1 is necessary for OCI-AML3 cells to maintain their leukemic phenotype. Drugs promoting mutant NPM1 nuclear localization are attractive candidates for clinical success in NPM1 mutated AML.

P174

THE LONG NON-CODING RNA HOXB-AS3 REGULATES RIBOSOMAL BIOGENESIS IN NPM1-MUTATED ACUTE MYELOID LEUKEMIA

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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-focus, has recently been associated with aggressiveness in several cancer entities. How-ever, the mechanistic role of HOXB-AS3 in this process remains uncertain.

Aims: Our aims were to evaluate the biologic significance of HOXB-AS3 expression in CN-AML, and to elucidate possible molecular mechanisms involved in HOXB-AS3 expression.

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 gapmers. 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 Basep-oc-CODEX (Advanced Cell Diagnostics, Newark, CA) according to the manufacturer’s instructions.

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 cells identified an lncRNA previously annotated (NR_033201/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 in NPM1mut pt blasts (n=5) led to a decrease 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 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 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 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 by OCI-AML3 cells in colony-forming assays (P=0.002).

Discussion: The discovery of HOXB-AS3 as a therapeutic target to compromise LSC function in IDHwtTET2wtAML patients, would be a critical therapeutic strategy to target LSCs. Further analysis of HOXB-AS3 expression in additional primary AML samples is warranted. The elucidation of HOXB-AS3 expression and functional mechanisms in AML and other hematological malignancies will offer new insights into the pathobiology of AML and the development of novel therapeutic strategies.

P173

NUCLEAR RE-LOCALIZATION OF NPM1C+ INDUCES DIFFERENTIATION AND CELL GROWTH ARREST

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Background: NPM1mut (NPM1c+) acute myeloid leukemia (AML) is a distinct entity of the 2016 WHO classification of hematopoietic tumors. NPM1mut is a multifunctional nucleolar chaperone. All the mutations in NPM1 described so far result in cytoplasmic protein localization (NPM1c) through the acquisition of a nuclear export signal (NES) at the C-terminus, indicating that the cytoplasmic localization is critical for the leukemic phenotype. The most frequent NPM1 mutation is a heterozygous 4bp insertion in exon 12 (mutA).

Aims: Use gene editing and targeted drug treatment to determine whether NPM1mut AML cells are dependent on the cytoplasmic localization of NPM1c. The results were validated in murine models by engrafting NSG mice with blasts of a NPM1mut AML pt.

Methods: Methods: We sought to introduce indels adjacent to the mutation to disrupt the C-terminal NES in the NPM1mut AML cell line OCI-AML3 and create novel edited alleles encoding for a mutant NPM1 with nuclear localization. Exploiting our optimized CRISPR-Cas9 protocol (Gundry et al., Cell Reports 2016), we designed the targeted editing of NPM1mut AML cells on drug treatment was studied using a CRISPR-Cas9 engineered NPM1c-GFP OCI-AML3 cell line grown in the presence of the nuclear hormone.

Results: While the NPM1mutA cell line showed 70-90% indel frequencies, the NPM1wt allele was intact. The novel edited alleles could direct nuclear localization of a reporter GFP-NPM1 fusion construct, and re-localization cytoplasmic NPM1 in edited cells was confirmed by immunofluorescence. We analyzed NPM1 protein expression by western blot and implementation of cell growth assays. We observed a fast and deep downregulation of the HOXA and HOXB cluster genes as well as MEIS1 in treated cells (4 to 5 fold average reduction). In order to verify that nuclear re-localization of NPM1c accounted for the dramatic changes, we treated OCI-AML3 cells with the nuclear export inhibitor selinexor (KPT-330). The impact of KPT-330 treatment mirrored the genome editing experiments, resulting in clear growth arrest, differentiation, and NPM1c localization with similar dynamics. Importantly, selinexor produced an almost complete loss of HOXA, HOXB and MEIS1 expression after 6 days of treatment.

Summary/Conclusions: Allele-specific editing is a powerful tool to probe non-genetic dependency. By achieving nuclear re-localization of mutant NPM1, we demonstrated that cytoplasmic localization of NPM1c is necessary for OCI-AML3 cells to maintain their leukemic phenotype. Drugs promoting mutant NPM1 nuclear localization are attractive candidates for clinical success in NPM1 mutated AML.
Summary/Conclusions: Conclusions: HOXB-AS3 is strongly associated with NPM1 mutations in AML. HOXB-AS3 interacts with ESBI 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 chemotherapy-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. S63845 and S55746) (MCL1, (Lessene et al., Nat Chem Biol, 2013) and MCL1 (Kotschy et al., Nature 2016). We hypothesize that simultaneous pharmacological targeting of BCL-2 and MCL1 will enhance apoptotic death of AML blasts, without increased toxicity to non-malignant cells.

Aims: To assess the feasibility and efficacy of targeting multiple BCL-2 pro-survival proteins using small molecule BH3-mimetics in pre-clinical models of AML.

Methods: AML cell lines were obtained from ATCC or DSMZ. S55746 (BCL-2 inhibitor) and S63845 (MCL1 inhibitor with 6-fold higher affinity to human than mouse Mcl1) were obtained from Servier and ATCC (BCL-2 inhibitor) from Guillaume Lessene (WEHI). Primary AML cells were obtained from patients providing informed consent. For in vivo experiments, NSG; NOD.Cg-Ptkd cyclodem1 Il2rgm1 Wjl/SzJ (NSG) or NOD/Rag2m1Il2rgm1Wjcr (NRRG) 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 10-1000-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. Bioluminescent 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 MDM2/3A. Patient-derived xenograft models 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 historical 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 cytoceduction of human AML cell line and primary AML samples in vitro and in vivo across a diverse range of AML genotypes. We provide preclinical support for the simultaneous dual pharmacological targeting of both BCL-2 and MCL1 represents a novel approach to treating AML without need for additional chemotherapy and with an acceptable therapeutic safety margin. Our 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 oncoprogen 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 RARa - mediated artificially by linking RARa to the dimerization domain of the NFκB 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 significantly increased the survival of mice transplanted with PmlC62A/C65A-p50-RARa, 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-p50-RARa leukemia as compared to PmlWT-p50-RARa, which is associated with detection of mutations found in human APL samples, including Ptpn11 and Usp9y. Using DNA repair reporter assays, we demonstrated that DNA repair via both non-homologous end joining (NHEJ; p=0.01) and homologous recombination (HR; p=0.006) pathways was less efficient in PmlC62A/C65A primary cells than in PmlWT cells. Importantly, using a PML-RARA-inducible cell line, comparable defects in the NHEJ and HR pathways, which were PML-RARa-dependent, were identified. These data were also supported by an increase in sister-chromatid exchange (p<0.0001) and chromosome abnormality (p=0.0002) rates in the context of PmlC62A/C65A versus PmlWT. Interestingly, the kinetics of repair of ionising radiation (IR)-induced DNA double-strand breaks, assessed by assessing γH2AX foci formation and clearance, was not affected. None of the DNA repair players analysed (e.g. Blm, Rad51 and 53BP1) failed to form foci in response to IR. However, their basal levels of foci were significantly greater in the presence of PmlC62A/C65A versus PmlWT. Furthermore, their basal levels of foci were significantly greater in the presence of PmlC62A/C65A versus PmlWT. Interestingly, the kinetics of repair of ionising radiation (IR)-induced DNA double-strand breaks, assessed by assessing γH2AX foci formation and clearance, was not affected. None of the DNA repair players analysed (e.g. Blm, Rad51 and 53BP1) failed to form foci in response to IR. However, their basal levels of foci were significantly greater in the presence of PmlC62A/C65A versus PmlWT.

Summary/Conclusions: Our study highlights the importance of re-formation of NBs for an efficient response to targeted therapy, the significant contribution
DECIPHERING THE ONCOGENIC NETWORK OF PRC2 LOSS GUIDED LEUKEMOGENESIS
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Background: Loss of function mutations in EZH2 (including the chromosomal abnormalities -7/-7a) and other PRC2 subunits have been identified in adults with MDS, MPN and AML. Moreover children with JMML and up to 30% of children with Down syndrome related AML present with mutations in PRC2 subunits. Since myeloid neoplasias are elicited by accumulation of cooperating mutations, we aimed to study the role of EZH2 loss in leukemic progression. We set out to decipher the oncogenic network guided by loss of PRC2-activity.

Aims: Through identification of collaborating mutations driving AML with loss of PRC2 function followed by molecular profiling we aimed to identify novel collaborating mutations.

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 quantitative genetic cooperation screenings was established. Four out of six CRISPR-Cas9 pools tested—comprised of five genes each and representing 148 mutation combinations—reproductively transformed LSK cells with distinct clonal output. Transplantation of in vitro immortalized clones yielded robust engraftment of mutant lineage 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 LSK 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 Nf1 (Ras-signaling), loss of Dnmt3a, 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 collaborating 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 vivo, 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 MULTI-LINEAGE 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 of Pmi 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.

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 Depth >10, Support Depth >20, SnpRupture >0.8, SiftScore <0.05, Gmaf <0.05, 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 transplanted bulk AML in NOD/SCID-IL2γnull (NSG) mice and analyzed human subpopulations (myeloid and lymphoid) of multi-lineage 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 patients with 1-18 mutations in a single clone (1-4 mutations) and a newly established 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 mutations.

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 mutations.

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 essential for the survival of MLL-rearranged 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 highly conserved NuA4 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 better understanding of the role of EPC1 and EPC2 in MLL AML biology would provide insight into the contribution of the homologous chromatin regulatory proteins EPC1 and EPC2 in AML in search for novel therapeutic targets.

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 Illumina sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChiPpeakAnno. Lentiviral supernatants were prepared and THP1 cells were infected with viral particles containing pLKO.1 puro lentiviral vector expressing shRNAs. 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, Ruvbl1, Mll-Af9) and loss of EPC1 and EPC2 in AML samples. To test the functional properties of pre-leukemic HSC in vivo, we transplanted bulk AML in NOD/SCID-IL2γnull (NSG) mice and analyzed human subpopulations (myeloid and lymphoid) of multi-lineage engrafted animals for the presence of leukemia-specific mutations.

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 mutations.
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 PRC2 target genes. RNA sequencing followed by gene-set enrichment analysis indicated significant transcriptional changes in PRC2 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 mono-acyclic 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 subgroup 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.
adult AML cases. Despite having poor outcomes, CK-AML is the least underst- 
stood 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 gene fusions that are not recurrent across patients, nonetheless deter- 
minate cancer genes that contribute to leukemogenesis in individual patients.

Methods: We performed a transcriptome analysis using Illumina paired-end (101bp×2) RNA sequencing of 65 CK-AML cases to identify gene fusions using mul- 
tiple independent algorithms (as paired reads that flank, or single-reads that span fusions). Fusions found in one CK-AML case were independently validated by array-based genomic profiling and/or long range PCR followed by use of long-read Oxford Nanopore sequencing technology.

Results: We identified 54 fusion gene 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. In addition to the 5' UTR, 3' Aix-Marseille University, 4Oncology, 5Cell therapy facility, Paoli-Calmettes Institute, 1Hematology, Paoli-Calmettes Institute, Marseille, 6Hematology, Toulouse Cancer University Institute, 22 nd Congress of the European Hematology Association

Figure 1. 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 than 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.

P183

H3K27ME3 LEVEL ON THE HIST1 CLUSTER: A POWERFUL EPIGENETIC 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 alter- 
ation 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 epigenetic biomarker corresponding to an abnormal gain of the repressive H3K27me3 histone mark within the HIST1 locus on the 6p22 referred as H3K27me3 HIST1high. Studying the diagnostic and therapeutic relevance of this epigenetic biomarker had an impact on clinical out- come as CN-AML patients with H3K27me3 HIST1high had a higher overall sur- 

Results: We analyzed 62 CN-AML samples from the training and validation sets together. Median age was 60 [37-76] and median leucocyte count was 76 G/L [10-352]. ChIP-QPCR Profiling identified 74 H3K27me3 HIST1high and 29 H3K27me3 HIST1low patients. FLT3-ITD was found in 33 (43%) of H3K27me3 HIST1high and 18 (38%) of H3K27me3 HIST1low patients. We confirmed that H3K27me3 HIST1high was associated with higher 5-year OS and LFS rates: 37% and 44% versus 17% and 19% (p=0.005 and .01) for the H3K27me3 HIST1high and the H3K27me3 HIST1low patients, independently of other genetic alterations. Combining our biomarker with FLT3 mutational status, we identified two subgroups of patients with very different outcome: 49% and 56% versus 18% and 18% (p=.004 and .01) for the H3K27me3 FLT3wt HIST1high and the FLT3mut H3K27me3 HIST1low patients, respectively (Figure 1). We performed GEP for 27 NPM1mut patients (12 H3K27me3 HIST1mut and 15 H3K27me3 HIST1mut). GSEA analysis revealed a strong enrichment in immune functions and leucocyte activation in the H3K27me3 HIST1high group, evoked differentiation AML. While H3K27me3 HIST1high samples had GSEA associated with chromatin remodeling factors and DNA replication. Considering only FLT3wt patients, the H3K27me3 HIST1mut subgroup had a gene expression signature characterized by a high expression level of genes from the HIST1 cluster which expression is known to be upregulated during S-phase of cell cycle.

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 D835 missense mutations in the kinase domain (KD) the predominant events. Sequencing of FLT3 in a cohort of 788 children with de novo AML treat- ed on Children’s Oncology Group (COG) protocols demonstrated that in addition to the previously described FLT3 mutations (ITD and D835), numer- ous 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 of FLT3, but varying 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 HEK293 cells using retroviral transduction. Following transduction, phosphorylation status of FLT3 (pFLT3) and downstream STAT5 (pSTAT5) were evaluated by immunoblotting. Phos- phorylation status was quantified by chemiluminescence analysis and the quan-
tently 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-ALM 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, 87% (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 and downstream demonstrated that in many 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 exclusively sensitive to crenolanib, with 9 of 10 mutations tested demonstrating an IC50 of pFLT3 inhibition ≤5.6 nM. Inhibition of downstream kinases are 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, M664I.

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 HYPOMETHYLATING AGENTS IN ACUTE MYELOID LEUKEMIA

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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 Keap1 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/Keap1 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 translocation 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 proteosomal 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.
**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 epigenome and chromatin accessibility levels between NRP/RPs 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.

**Method:** FLT3 inhibition overcomes resistance to the BCL-2 selective antagonist, BCL-2 in AML patients and BCL-2 selective agents like venetoclax show promising clinical activity in refractory AML patients.

**Results:** In vitro, FLT3 inhibition overcomes resistance to the BCL-2 selective inhibitor, venetoclax. FLT3 inhibition synergizes with venetoclax to overcome resistance to BCL-2 selective inhibitors in AML cell lines.

**Conclusion:** The combination of FLT3 and BCL-2 selective inhibitors offers a promising therapeutic approach for the treatment of AML patients with resistant disease.

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**References:**

2. A. Souers, J. Leverson
3. A. Souers, J. Leverson, A. Austen, C. Fiorenzo, E. Lee, D. Smith, C. Fritz, T. Lodie, E. Tomaso, E. Ols
4. A. Souers, J. Leverson
5. A. Souers, J. Leverson

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**Conclusion:** The use of FLT3 inhibitors in combination with BCL-2 selective agents like venetoclax shows promising clinical activity in refractory AML patients.

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**References:**

2. A. Souers, J. Leverson
3. A. Souers, J. Leverson, A. Austen, C. Fiorenzo, E. Lee, D. Smith, C. Fritz, T. Lodie, E. Tomaso, E. Ols
4. A. Souers, J. Leverson
5. A. Souers, J. Leverson

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**Conclusion:** The combination of FLT3 and BCL-2 selective inhibitors offers a promising therapeutic approach for the treatment of AML patients with resistant disease.

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**References:**

2. A. Souers, J. Leverson
3. A. Souers, J. Leverson, A. Austen, C. Fiorenzo, E. Lee, D. Smith, C. Fritz, T. Lodie, E. Tomaso, E. Ols
4. A. Souers, J. Leverson
5. A. Souers, J. Leverson

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**Conclusion:** The use of FLT3 inhibitors in combination with BCL-2 selective agents like venetoclax shows promising clinical activity in refractory AML patients.
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 IGFBP7low 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

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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1MD Anderson Cancer Center, Houston, TX, 2H. Lee Moffitt Cancer Center, Tampa, FL, 3Dana-Farber Cancer Institute, Boston, MA, 4City of Hope National Medical Center, Duarte, CA, 5The Ohio State University, Columbus, OH, 6Duke University Medical Center, Durham, NC, 7Roswell Park Cancer Institute, Buffalo, NY, 8Sterling Therapeutics, New York, NY, United States

Background: SL-401 is a targeted therapy directed to interleukin-3 receptor a (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: 0.5-22.9 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 for 18 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 consists largely of transaminitis and thrombocytopenia. CLS can be fatal. Side effects have generally tended to decrease in frequency and severity with increasing cycles. Updated data, including detailed safety analysis across all ongoing SL-401 studies will be presented at the meeting.

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
Table 1.

<table>
<thead>
<tr>
<th>Genes</th>
<th>MLCL (%)</th>
<th>MCLC (%)</th>
<th>CMC (%)</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>5404TA</td>
<td>31%</td>
<td>19%</td>
<td>14%</td>
<td>CHIP-assisted</td>
</tr>
<tr>
<td>5404TA</td>
<td>100%</td>
<td>99%</td>
<td>98%</td>
<td>39%</td>
</tr>
<tr>
<td>TP53</td>
<td>15%</td>
<td>9%</td>
<td>5%</td>
<td>RTK pathway</td>
</tr>
<tr>
<td>5404TA</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>88%</td>
</tr>
<tr>
<td>TP53</td>
<td>24%</td>
<td>10%</td>
<td>9%</td>
<td>ErbB Family</td>
</tr>
<tr>
<td>5404TA</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>89%</td>
</tr>
<tr>
<td>CEBPA</td>
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<td>0%</td>
<td>0%</td>
<td>Chromatin-Dependent</td>
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<tr>
<td>5404TA</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>67%</td>
</tr>
<tr>
<td>ID2</td>
<td>38%</td>
<td>44%</td>
<td>38%</td>
<td>Splicing</td>
</tr>
<tr>
<td>5404TA</td>
<td>33%</td>
<td>17%</td>
<td>17%</td>
<td>33%</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.

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 alloHSCT rates 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 RUNX1 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. RUNX1 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 6 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 patients with germline mutations. In 10 (48%) of 21 patients with germline mutations but not in somatic mutations, the missense mutation p.L56S was not found. In 13 (62%) of 21 patients with germline mutations, there was a 0.96 (CI=0.82-1.12), low 1.06 (CI=0.88-1.27).

44 | haematologica | 2017; 102(s2)
Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR (95% CI)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>ApoC4 (mg/dL)</td>
<td>1.00 (0.97-1.02)</td>
<td>0.42</td>
</tr>
<tr>
<td>TPS (IU/L)</td>
<td>1.00 (0.98-1.03)</td>
<td>0.84</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>1.00 (0.97-1.03)</td>
<td>0.70</td>
</tr>
<tr>
<td>IDH1 mutation group</td>
<td>1.00 (0.89-1.11)</td>
<td>0.70</td>
</tr>
<tr>
<td>RUNX1 mutation group</td>
<td>1.00 (0.89-1.11)</td>
<td>0.70</td>
</tr>
<tr>
<td>NPM1 mutation group</td>
<td>1.00 (0.89-1.11)</td>
<td>0.70</td>
</tr>
<tr>
<td>FLT3 mutation group</td>
<td>1.00 (0.89-1.11)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

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.

Figure 1.
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 other myeloid malignancies.

**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 in 21 of these genes, 5 structural variant (SV) breakpoints in 3 genes were also targeted. Barcoded libraries were sequenced with the MiSeq® platform and analyzed using proprietary Invivoscribe (IVS) MyInformatics™ 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 (≤ 50%). 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). In addition, using clinical samples the MyMRD assay shows excellent concordance with the standard FLT3 CE assays for variants with VAFs above the CE detection threshold (5%). Sanger sequencing was the only method actually covering the entire coding region of CEBPA. Both NGS amplicon-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.

**P198**

**IS IT POSSIBLE TO RELIABLY DETECT CLINICALLY-RELEVANT BIALLEIC CEBPA GENE MUTATIONS USING NGS PANELS?**


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**Background:** CEBPA gene encodes a leucine zipper transcription factor that is important for normal myeloid cell differentiation. Biallelic CEBPA (bicEBPA) mutations are associated with favourable prognosis in patients with acute myeloid leukaemia (AML); therefore, accurate molecular testing of this gene is crucial in the clinical setting. Molecular pathology labs routinely analyse CEBPA through fluorescence-based multiplex-PCR fragment analysis or, more frequently, Sanger sequencing. Lately, it is increasingly common to use next-generation sequencing (NGS) technology in the pathology labs, and CEBPA gene is indeed included in the majority of NGS panels commercially available for testing of patients with neoplasias.

**Aims:** We set ourselves to compare the performance of two different NGS targeted panels against bicEBPA molecular aberrations, with a particular focus on bicEBPA mutations.

**Methods:** DNA specimens from 173 myeloid cases were subjected to Sanger (n=92) or to NGS (n=81) sequencing, including the TruSight Myeloid Sequencing Panel (illumina) (n=59), and the IonAmpSeq AML Community Panel (thermax Fischer) (n=22). Cases showing two variants were further analysed through cloning of the whole length of CEBPA and subsequent Sanger sequencing of at least 10 colonies from each case.

**Results:** We called 10 CEBPA variants affecting 7 samples through NGS. Both NGS panels are designed to cover CEBPA through overlapping amplicons (e.g. FLT3). However, we found that among the set of 3.5 amplicons were covered <500x, and more worryingly, we realised that at least one of those amplicons was shallowly (<100x) covered in 97% of the cases. Indeed both panels showed significantly lower average coverage levels of this gene compared to the panel as a whole (Figure 1). This might not be surprising, since CEBPA is not included within a CpG-rich region, and therefore its amplification needs tailored PCR conditions, hard to address in the multiplexed PCR step included in their library prep protocols. Therefore, both NGS approaches are prone to miss variants. In contrast, Sanger sequencing protocol (which includes optimized PCR conditions for correct amplification of the CEBPA gene) managed to cover the whole length of the gene. We were able to detect 26 variants affecting 20 AML cases through Sanger sequencing. Cases showing two variants were manually curated (through Chromos or IGV tools) to confirm if they affected different alleles. However, in 6 cases both mutations layed on different amplicons, which made not possible to univocally conclude if and how they were biallelic. These inconclusive cases were subjected to DMSO-Pfu-PCR in order to amplify the whole length of CEBPA coding region, followed by cloning. Colony sequencing showed independent clones harbouring different variants (i.e. bona fide bicEBPA mutations) in the majority of the cases, but crucially, not in all of them. This result highlights the need of implementing techniques able to accurately assess CEBPA biallelism, unless than plain calling of more than one variant.
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 \textit{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 \textit{RUNX1-RUNX1T1} AML outside of clinical studies. Defining MRD levels by \textit{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 \textit{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

NUMBER OF TP53 ABNORMALITIES AND THEIR CLINICAL RELEVANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASIA

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Background: Mutations in TP53 can be detected in up to 16-19% 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. TP53 mutations were less likely to have co-occurring chr17 abnormalities in patients with TP53 mutations than patients without TP53 mutations (25%, n=146) compared to AML (19%, n=154) (p=0.012) with 251 (13%) 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 mutation was detected. Correlation between TP53 mutations and deletions (p=0.443, p<0.001) was observed with 172 (12%) patients having 1 TP53 abnormality. 169 (12%) patients 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).

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: RESULTS FROM A Phase 1 study

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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. Durations of remissions 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 13/58 (22%) responding patients achieved minimal residual disease (MRD) negativity (F Ravandi, MD, unpublished data, Jan 2017). Vadastuximab talirine (SGN-CD33A; 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: 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 regimen], standard dosing). CRi required either platelet count 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 with 33A+HMA. Patients had adverse (38%) or intermediate (62%) cytogenetics (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 in 55%, febrile neutropenia (49%), anemia (46%), neutropenia (42%), pneumonia (16%), and leukopenia (7%); 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 (43%), constipation (43%), peripheral edema (43%), dyspepsia (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 (≥74%) were maintained across adverse disease subsets including advance cytogenetics (16/18, 89%), TP53-mutated (67/86), 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-HSCT, and no SOS/VOD was observed. The median post-MRD clearance rate among responding patients who received 33A+HMA is higher than the rate observed with single

Figure 1.
Background: Acute myeloid leukemia (AML) with intermediate-risk (IR) cytogenetics includes a substantial proportion of patients with favorable molecular profile (FMP); in which AML cells harbor the NPM1 mutation or CEBPα 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 191 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 high-dose cytarabine (HDAC) and, depending on the protocol, additional HDAC, autologous or allogeneic 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 19x10⁹/μL (range 0.55-282). One-hundred eighty-two patients had a normal karyotype and it was abnormal in 34 (5 patients no metaphases). There were 52±8% and 70±4%, respectively. In univariate comparisons, better EFS and OS was observed in CEBPα+/FLT3-ITD- patients compared to those with NPM1+/FLT3-ITD- (p=0.03 and p=0.02, respectively). When analyzing post-remission treatment, patients treated with HDAC only had an excellent prognosis, even better than those receiving an autologous or allogeneic transplantation. One patient died in CR in the HDAC group, another in the autologous transplant group and 7 in the allo-HCT group (p<0.001). Death during induction was observed in 16 patients (7%), all of them with NPM1+/FLT3-ITD+. Induction results according to age were similar in both groups. Event-free survival and overall survival are reported at 8 years and were 52±8% and 70±4%, respectively. In univariate comparisons, better EFS and OS was observed in CEBPα+/FLT3-ITD- patients compared to those with NPM1+/FLT3-ITD- (p=0.03 and p=0.02, respectively). When analyzing post-remission treatment, patients treated with HDAC only had an excellent prognosis, even better than those receiving an autologous or allogeneic transplantation. One patient died in CR in the HDAC group, another in the autologous transplant group and 7 in the allo-HCT group (p<0.001). In multivariate analysis of pretransplant characteristics, age up to 60 years and CEBPα+/FLT3-ITD- associated to improved EFS (RR=0.42) and OS (RR=0.29). Interestingly, in a subgroup of 123 patients with data on MRD after consolidation chemotherapy (flow citometry, cut-off: 0.12%), positivity was associated with worse EFS (0.02). Despite age was a prognostic factor, patients older than 60 years with IR-FMP AML had remarkable EFS of 36±3% and OS 54±10% at 8 years (Figure 1).
Results: 24 pts have been enrolled to date and 17 are evaluable for response. The median age was 60 years (range, 50-70) 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 (13%), pneumonia (20%), cardiac edema (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 sAML in Phase 1 (DeAngelo, EHA 2016); no autoimmunization or evidence of drug-drug interactions were apparent. The median E-sel ligand expression at baseline was 29% (range, 2-67%) of blasts in the bone marrow.

Summary/Conclusions: The addition of a novel E-selectin antagonist, GMI-1271, to anthracycline 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 is 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. CR was the key secondary endpoint.

Methods: Pts suitable for IC (ECOG PS 0-1, creatinine ≤1.3 mg/dL, no severe cardiac disease) gave informed consent and received glasdegib 100 mg QD from day 1, in combination with cytarabine 100 mg/m² i.v. for 7 days and daunorubicin 60 mg/m² IV for 3 days, followed by 2-4 consolidation cycles (cytarabine 1 g/m² Q12 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 IC (2 pts not treated due to inability). Among MDS pts, 19 pts (47%-de novo, secondary) were distinguishable, 32% int/med, 21% int/IIl and 26% adverse cytogenetic abnormalities (1 pt not assessed). Among MDS pts (5 de-novo), 20 had good, 40% int and 40% poor risk cytogenetic abnormalities. Median age was 64 (27-75) years. Median treatment duration was 48 (10-502) days. The most common NCI-CTC v4.0 Grade 3/4 AEs included: hypokalemia (13%), hyponatremia (11.6%) and hypertension (10.1%). Grade 5 AEs within 28 days from last dose (5 pts, 7.2%) included pneumonia, sepsis, septic shock (1 pt each) and disease progression (2 pts). The observed steady-state plasma exposures for glasdegib were as expected at the 100 mg dose level. Based on investigator’s assessment, CR was 41% (80% CI 33.2-47.9) and CRi was 49%. CR for good/int. risk pts (n=49) was 49% and for poor risk pts (n=19) 21%. Forty-one (59%) pts died (33 [48%] due to disease progression) with median follow up of 30.1 months; 24 (35%) remain in follow up. The median OS (mOS) was 14.9 months (80% CI 12.0-17.3) for AML pts and 25.1 months (90% CI 19.3-30.9) for MDS pts. After a median of three (33%) pts received a transplant and MDS censored for transplant was 17.7 months, AML Pts >60 yrs. The mOS for 44 AML pts age ≥60 yrs is presented in the Table 1, in the context of historical control.

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 Rollig et al, 2011) months</th>
<th>ICT + Glasdegib (n=44) months</th>
<th>Increase in mOS (%) (29 events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>14.6</td>
<td>Not reached (n=9)</td>
<td>Not estimable</td>
</tr>
<tr>
<td>Int-I</td>
<td>15.7</td>
<td>13.7 (n=12)</td>
<td>65.3</td>
</tr>
<tr>
<td>Int-II</td>
<td>9.2</td>
<td>13.4 (n=12)</td>
<td>45.7</td>
</tr>
<tr>
<td>Adverse</td>
<td>4.8</td>
<td>3.5 (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 IC, the mOS for AML pts >60 yrs stratified by subgroup compares favorably by adding glasdegib. Our data can be used to support that this is a rational combination of glasdegib on the leukemia stem cell. 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.

P205

CM942 IS A NEW SMALL MOLECULE THAT TARGETS SET-PP2A INTERACTION 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. Our group showed that the inactivation of the tumor suppressor PP2A is a recurrent event in AML, and that overexpression of SET, an endogenous inhibitor of PP2A, is a poor prognostic factor in this disease. Furthermore, the anticancer activity of FTY720, a PP2A-activating drug (BA266), depends on its interaction with SET, FTY720 is a relatively non-toxic drug currently used in patients with relapsing multiple sclerosis; however, this drug cannot be used in cancer patients due to its toxicity at the needed anti-neoplastic dose. Therefore, investigation of alternative agents for reactivation of PP2A is warranted.

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 PP2A 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 phosphosylation of the PP2A target 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. By western blot analyses we found that those patients responding to CM942 treatment had SET overexpression. Of note, treatment of peripheral blood mononuclear cells from healthy donors with CM942 had no effects on cell viability. Therefore, although FTY720 and CM942 have similar effects inhibiting cellular proliferation, CM942 was less toxic when assayed on normal peripheral blood cells.

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 FTY20 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 in combination with other drugs, will cure the disease, or allow for a cure in the future with conventional chemotherapy. Our results indicate that PADS may be a valid therapeutic option for AML, especially for treating leukemias characterized by SET-dependent inactivation of PP2A.
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 relapse, 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 specific mutations were detected (1-18 mutations/AML). As expected, these included recurrently mutated genes like DNMT3A (in 4 patients), IDH1 and 2 (each in one patient), KIT and NRAS (both in 2 patients). Categorization of identified mutations showed that these mutations affected genes involved in various cellular processes including transcriptional regulation (15), cell differentiation (6), cell cycling (5), apoptosis/survival signals (5), proliferation (3), cell growth (3) and splicing (3). Empirical Bayesian clustering of all detected variants according to their respective AF resulted in 2-5 different clusters per AML.

Based on this cluster analysis we were able to predict the founding clusters/clone. Assuming that most of the mutations are heterozygous and considering the blast percentage at diagnosis, mutations of the biggest clusters are involved in phenotypically distinct subpopulations and during xenotransplantations by targeting sequenced. An update of this analysis will be presented at EHA.

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 ≤15x10^9/L received AZA (75mg/m^2x7 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%–12.3% 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**

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**Background:** Fludarabine and clofarabine are purine nucleoside analogues with clinical activity in 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 FLT3-ITD mutations could receive concomitant sorafenib. Responding pts could receive up to 6 cycles of consolidation at attenuated doses. Outcomes were compared 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 (53%) in the FIA arm received sorafenib. The composite CR/CRI 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 multiparameter 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 12 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/CRI rates, EFS or OS between the two groups. However, among pts <50 years of age, the median EFS for pts who received FIA (n=38), CIA (n=28) and IA (n=34) was not reached. 10 months and 3 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:** CIA and FIA were safe and well tolerated. CIA was found to be associated with better efficacy outcomes as compared with FIA, especially 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 TRANSPORTATION TO INFECTION 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:** Approximately 20% to 30% of patients with acute myeloid leukemia (AML) have FLT3 mutations; these patients often experience rapid post-induction relapse, highlighting the need for therapies that provide an improved bridge to stem cell transplantation. CPX-351 is a liposomal formulation that delivers 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 (Lordan, 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/day [1st induction: x 7 days] or 100 mg/m²/day x 7 days at 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 study 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/myelodysplastic syndrome (MDS) (19%) or without (10%) prior therapy; myelodysplastic syndromes (MDS) (19%) or therapy as a result of non-randomized single-agent; AML after chronic myelomonocytic leukemia (12%); and de novo AML with MDS karyotype (21%). In FLT3+ patients, median OS was longer with CPX-351 than with wild type FLT3 (Lordan, et al. Leuk Res. 2017;53:39-49).

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

The adverse event profile (reported during treatment or within 30 days of discontinuation of study treatment) in CPX-351 in FLT3+ patients was consistent with the overall study population. Serious treatment-emergent adverse events (TEAEs) were experienced by 7 (32%) FL3+ 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 n=2 in 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 haematologica | 2017; 102(s2) | 53
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.

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.

Figure 1.

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 re-induction or maintenance therapies with 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 patients 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 and treated on the trial. Baseline characteristics are outlined in Table 1. AML-related mutations detected at start of therapy include: IDH2 (n=2), NPM1 (2), TET2 (2), and 1 each of TP53, JAK2, ASXL1, and DNMT3a. High risk features at the time of enrollment were as follows: 2 (25%) persistent MRD, 2 (25%) adverse karyotype, 1 (13%) adverse mutualation profile, and 3 pts (38%) in CR2 or beyond. Pts have received a median of 4 (1 - 13) cycles of therapy. With a median followup of 6+ months (1 – 14), the 6- and 12-month estimated RFS were 88% and 73%, respectively. The 6- and 12-month estimated OS were 100% (Figure 1). The one patient who died was discovered after enrollment to actually be in CR4. This patient relapsed approximately 8 months after achieving CR4. The regimen was well tolerated overall, with 4 pts having possible immune-related events. 1 pt patient had grade 3 thyroiditis leading to hypothyroidism, treated successfully with steroids and thyroid hormone supplementation, who continues on treatment. 1 pt had grade 4 transaminase elevation which responded to dose interruption and who continues on treatment. 2 pts had grade 3 possible pneumonitis treated successfully with steroids and dose interruption – both of whom continue on treatment (Table 1).

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

Figure 1.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range)</th>
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<tbody>
<tr>
<td>Age</td>
<td>60 (56 - 71)</td>
</tr>
<tr>
<td>WBC [10^12/L]</td>
<td>3.8 (1.3 - 8)</td>
</tr>
<tr>
<td>Platelets [10^12/L]</td>
<td>125 (72 - 272)</td>
</tr>
<tr>
<td>ALAT [U/L]</td>
<td>43 (17 - 69)</td>
</tr>
<tr>
<td>Albamin</td>
<td>3.9 (4.0 - 6.0)</td>
</tr>
<tr>
<td>Hb</td>
<td>11.9 (9.6 - 15.2)</td>
</tr>
<tr>
<td>Creatinie</td>
<td>0.7 (0.5 - 0.8)</td>
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</tbody>
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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 (lncRNAs) are non-protein coding RNAs longer than 200 nucleotides. Recently, a number of lncRNAs 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 TPS3 mutation (p=0.011).

Summary/Conclusions: Higher expression of KIAA0125 in AML patients was correlated with mutations of RUNX1, DNM3TA, 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- and a correlation of their expression with LSC signatures might in part explain its unfavorable impact on the survival of AML patients.

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 (86.6%) 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).

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 subgroups 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 HCT (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: Patients with baseline WT1 <250 copies were excluded. All patients underwent intensive induction chemotherapy with curative intent and subsequent consolidation chemotherapy according to the AML risk assessment (autologous peripheral stem cell transplantation for low risk and allo-HCT for intermediate and high risk patients). Effect of post induction WT1 expression on disease-free survival (DFS) and overall survival (OS) and of pre allo-HCT WT1 expression on relapse (R) rate were investigated.

Results: Baseline BM WT1 expression were not found significantly associated with demographic, clinical and disease biological features at diagnosis. Baseline BM WT1 expression lacked even to show an association with response to induction chemotherapy (OR 1.16; 95% CI 0.90-1.50, p=0.244). OS and DFS were significantly shorter in first CR with >350 WT1 copies after induction compared to those with ≤50 (OS 17 vs 95 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 (NRI=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-adjusted 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. Prospective 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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1Massachusetts General Hospital Cancer Center, 2Harvard Medical School, Boston, 3The University of Texas MD Anderson Cancer Center, Houston, 4Cellgene Corporation, Summit, 5Agios Pharmaceuticals, Inc, Cambridge, 6Memorial Sloan Kettering Cancer Center, 7Weill Cornell Medical College, New York, United States, 8Institut Gustave Roussy, Villejuif, France

Background: Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 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, fully functional neutrophils (Yen et al., Cancer Discov, 2017). 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 pts treated with 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).

Summary/Conclusions: The presence of SC is a prognostic factor in terms of response, OS and EFS. Accordingly, SC detection could help to identify prognosis subgroups of patients with different prognostic among those in the intermediate risk group by genetics/molecular assays.
Methods: An independent Differentiation Syndrome Review Committee (DSRC) was formed to review potential cases of IDH-DS. The DSRC identified and agreed upon a series of signs and symptoms possibly characteristic of IDH-DS, including fever, lung infiltrates, pleural or pericardial effusions, rapid weight gain, edema, and azotemia. In all, 27 cases (8 of investigator-reported IDH-DS and 19 with characteristics suggestive of IDH-DS) were identified and retrospectively reviewed by the DSRC to determine their consistency with IDH-DS.

Results: The DSRC determined 13 cases (11.9% of 109 R/R AML pts in the enasidenib 100 mg/day dosing cohort) to be consistent with IDH-DS. Median time to onset was 30 days (range 7-116). Manifestations of IDH-DS in >2 pts were dyspnea (n=10), pyrexia (9), lung infiltrates (8), pleural effusion (5), and kidney injury (3). IDH-DS was effectively managed with systemic corticosteroids in 12/13 cases. Leukocytosis accompanied 4/13 cases, for which hydroxyurea was employed for cytoreduction. Enasidenib was interrupted for 9 pts (for a median of 7 days), but dose reductions or enasidenib discontinuation were not required for pts with IDH-DS. Six of the 13 pts had clinical responses (2 complete remissions [CR], 2 CRs with incomplete hematologic recovery, 1 partial remission, and 1 morphologic leukemia-free state), 6 pts had stable disease, and 1 pt had progressive disease.

Summary/Conclusions: Systemic corticosteroids, close hemodynamic management, and hydroxyurea (in the presence of leukocytosis) are effective IDH-DS management strategies; they should be administered promptly when IDH-DS is suspected, and continued until improvement. Enasidenib interruption can be considered if initial intervention is unsuccessful. IDH-DS represents a novel clinical finding in pts with mutant AML treated with enasidenib, and is likely due to its suggested mechanism of action, myeloblast differentiation.
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.

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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versity Hospital, Hradec Kralove, 4University Hospital, Olomouc, 5University Hospital Kralovske Vinohrady, 6CLSG Data Center, Prague, 7University Hospital, Pilsen, 8University Hospital Motol, Prague, Czech Republic

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

Aims: The objective of this study is to analyze elderly DLBCL patients prospectiv-
ely registered in NIHIL 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. Diagnose, 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 pathol-
ogy 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-Maier 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 high or inter-
mediate high iPI 49.1%, with bulky disease (>10 cm) 17.0%, with lower albumin 27.7%, with Charlson Comorbidity Score (CCS) ≥4 25%. According to treatment choice of physician (intent to treat), pts. could be divid-
ed into 3 groups R-CHOP (CH), cyclophosphamide (CF) 750 mg/m2and A 25
mg/m2, (Peyrade 2011) or modified R-CHOP (modiCH) (CF 750 mg/m2and A 25
mg/m2for any other dose between CHOP and miniCHOP). There were 21 pts. (18.8%) treated with CH, 38 (33.9%) with miniCH and 53 (47.3%) with modiCH. 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 comorbidity (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), 5 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
well as PFS) compared to stable or progressive disease with 4.6 vs 3.5 y vs
0.8 y 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 minor-
ity of these pts full dose of R-CHOP could be safely used and there is trend to
better overall survival.

Supported by AZV 16-31092A.

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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Comprehensive Cancer Organisation (IKNL), Utrecht, Netherlands

Background: PCNSL is a rare, aggressive form of an extranodal non-Hodgkin lymphoma that exclusively affects the CNS. Recent findings from the few avail-
able 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 nation-
wide 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 inci-
dance rates (ASR) were calculated per 1,000,000 person-years and standard-
ized 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 1,873 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 appli-
cation 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 RT 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 ther-
apy 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
therapy. But disease-free survival in DLBCL patients was significantly worse.

**P220** CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF DIFFUSE LARGE HEPATITIS C - ASSOCIATED NON-HODGKIN LYMPHOMA (DLBCL+C)

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1Russian National Research Medical University named after N.I. Pirogov, 2National Research Center for Haematology, 3National Research Center for Oncology named after N.N. Blochin, 4City Clinical Hospital named after C.P. Botkin, 5City Clinical Hospital named after V.M. Buyanov, Moscow, Russian Federation

**Background:** In the WHO classification (2008), hepatitis C virus distinguishes as one of the etiological factors of multisite etiopathogenesis DLBCL. 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:** Patients’ 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 1.3 in patients with DLBCL+C, 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). Extranodal lesions detected in 72% in DLBCL+C and in 26% in C DLBCL-C (p=0.006). In comparable groups localization of extranodal lesions was: spleen (52% to 23%), bone marrow involvement (43% and 27%), liver (26% and 14%); GCB / non-GCB histological variants ratio GCB / non-GCB in DLBCL+C; 36% / 64% in DLBCL-C group GCB / non-GCB (p=0.001). Hepatitis C virus RNA in blood was detected by PCR. Viral RNA was found in 78% (74 patients). High viral load was in 21% of patients. In 22% of cases markers of hepatitis C virus in blood were identified by ELISA. All patients received chemotherapy according to the scheme CHOP / R-CHOP. The frequency of complete remission was 60% in the group of patients with DLBCL+C and 63% of DLBCL-C. Median overall survival (OS) was 46 months in group DLBCL+C and 71 months in DLBCL-C (p=0.0003). Median progression-free survival (PFS) was 28 months in DLBCL+C 47 months in the control group (p=0.0002). According to the immunohistochemical variant of DLBCL: GCB (46%), non-GCB (54%) in GCB and non-GCB in DLBCL+C (p=0.002). Median PFS was 36 and 47 months in comparable group. Median OS was 18 months in non-GCB DLBCL+C and 70 months in non-GCB DLBCL-C (p=0.00001). Median PFS groups was 13 and 42 months, respectively. 56 patients received antiviral therapy after chemotherapy. Median OS was 63 months in GCB DLBCL+C with antiviral therapy and 28 months in GCB DLBCL-C without antiviral therapy (p=0.0002). Median PFS was 46 and 20 months, respectively. Median OS was 22 months in non-GCB DLBCL+C with antiviral therapy and 17 months in non-GCB DLBCL-C without antiviral therapy. Median PFS in the group was 11 and 15 months, respectively.

**Summary/Conclusions:** DLBCL+C characterized by aggressive course of the disease (younger age at onset of the disease, advanced stages, extranodal involvement), which is one more evidence possibility of separating DLBCL+C in a separate group. Although there is no difference in the effectiveness of the therapy. But disease-free survival in DLBCL patients+C was significantly worse.

**P221** MAGNETIC RESONANCE IMAGING FOR EARLY DETECTION OF ANTHRACYCLINE CARCINOToxICITY IN MALIGNANT LYMPHOMA

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**Background:** Doxorubicin is a cornerstone of curative chemotherapy for lymphoma. However, doxorubicin therapy is limited by cardiac side effects including high-mortality heart failure (HF). Signs of cardiotoxicity often appear too late to avoid irreversible myocardial damage.

**Aims:** The aim of our study is to investigate the value of rubidium 82 positron emission tomography (82Rb PET), ictus 123i metiodobenzoyguanidin (123I MIBG) and cardiac magnetic resonance (MR) imaging in early detection of doxorubicin-induced cardiomyopathy and prediction of HF in patients with malignant lymphoma. We aim to identify early signs of cardiotoxic injury that predict the formation of interstitial fibrosis and subsequent HF. Here we present our preliminary MR data. 82Rb PET and 123I MIBG data will be analysed later.

**Methods:** 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 (SV) (p=0.02). However, from the acute MR to follow-up 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.

**P222** Abstract withdrawn.

**P223** RELAPSE CHARACTERISTICS AND THE ROLE OF SURVEILLANCE COMPUTED TOMOGRAPHY IN AGGRESSIVE NON-HODGKIN LYMPHOMA

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Results: The use of surveillance computed tomography (CT) is usual practice for cases of complete remission (CR) in aggressive non-Hodgkin lymphoma (aNHL). However, there is a lack of evidence to support this strategy.

Aims: To determine whether surveillance CT could contribute to the improvement of survival in relapsed aNHL patients, we retrospectively analyzed our institutional lymphoma registry, which enrolled consecutive patients with lymphoma from June 1995 to October 2016. Of 1,385 aNHL patients in the registry, 664 patients achieved CR and received follow-up through clinical visits, with or without surveillance CT.

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 interval were at the discretion of the treating physicians.

Methods: Relapse was detected in 171 patients, of whom 152 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 either the improvement of 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, 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%) who achieved CR after salvage chemotherapy (CR2). 37 (88%) patients 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 46.5 months, p=0.182) (Table 1).

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.

Table 1.

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<th>Characteristics at relapse according to method of detection</th>
<th>CR Group 1 (n=37)</th>
<th>CR Group 2 (n=99)</th>
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Discussion:

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Figure 1.

Summary/Conclusions: GLIDE is an effective regimen for newly diagnosed stage IV and relapsed ENKTL. Up-front ASCT after achieving CR can reduce relapse and prolong survival. Treatment related adverse reactions and support care need concerns.

P224

A MULTI-CENTER STUDY OF GLIDE CHEMOTHERAPY CONSOLIDATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR NEWLY DIAGNOSED STAGE IV AND RELAPSED EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMAS PATIENTS

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Background: The prognosis of advanced-stage and relapsed extranodal NK/T cell lymphoma (ENKTL) is poor, with long term survival rate of 30%. Our previous study of GLIDE (gemcitabine, L-asparaginase, ifosfamide, dexamethasone and etoposide) chemotherapy reported complete response (CR) rate and 3-year overall survival (OS) of these patients were 57.1% and 56% respectively. We assumed autologous stem cell transplantation may further improve the prognosis of these patients.

Aims: We conducted this clinical trial to address the efficacy and safety of our treatment strategy, GLIDE induction followed by ASCT, in newly diagnosed stage IV and relapsed ENKTL.

Methods: We treated 60 patients with newly diagnosed stage IV (n=49) and relapsed (n=11) ENKTL from 2010 to 2016. The median age at recruitment was 38 years and the median follow-up period was 13.4 months. Patients were treated with GLIDE (gemcitabine 800 mg/m2 D1,5; L-asparaginase 6000 U/m2 D4,6,8,10,12 or peg-asparaginase 2500 U/m2 D4,11; ifosfamide 1000 mg/m2 D1-3; dexamethasone 20mg D1-4; etoposide 100 mg/m2 D1-3) every 4 weeks, and responses were evaluated with PET/CT every 2 cycles. Patients achieving CR underwent ASCT or continued with GLIDE up to 6 cycles. Others finished 6 cycles of GLIDE. Overall response rate (ORR), CR, OS and progression free survival (PFS) were calculated using standard methods. Statistical analysis was done using Fisher’s exact test or Chi-square test / Kruskal-Wallis test. Kaplan-Meier method was used for time-to-event analysis including overall survival and progression free survival. The Log-rank test was used to evaluate the difference in time-to-event endpoints between patient groups.

Results: Fifty-seven patients had finished planned treatment with 1 withdraw of informed consent after cycle 1, and 2 death of sepsis during cycle 1 and cycle 2 respectively. Twenty-one patients underwent ASCT. The ORR was 81.4% and the CR was 69.5% with early CR (CR after 2 cycles) of 57.6%. Estimated 5-year OS and PFS rates of the whole cohort and patients underwent ASCT were 68.7%, 54.0%, 79.6% and 85.2% respectively. Univariate analysis revealed that ECOG ≤1, IPI ≤2, early CR and ASCT were associated with less relapse and death. Multivariate analysis showed ECOG ≤1 was an independent risk factor for disease progression (HR=4.321, 95% CI: 1.127–16.572, P=0.033) and death (HR=4.256, 2.150–993.190, P=0.014) and ASCT was associated with better PFS (HR=0.058, 95% CI: 0.007–0.495, P=0.009) and OS (HR=0.019, 95% CI: 0.001–0.596, P=0.024). Figure 1 highlights the OS and PFS of whole cohort (A) and ASCT patients (B). Myelosuppression was the most common adverse reaction (AE). The incidences of grade 3-4 adverse reactions were as follows: myelosuppression, 46.6%, 28.6% and 5.3% respectively. The most common non-hematologic AE was fever with neutropenia (36.5% of total cycles), while others were mild and manageable.

Figure 1.

Summary/Conclusions: GLIDE is an effective regimen for newly diagnosed stage IV and relapsed ENKTL. Up-front ASCT after achieving CR can reduce relapse and prolong survival. Treatment related adverse reactions and support care need concerns.

P225

LONG TERM FOLLOW-UP OF PATIENTS WITH PERIPHERAL T-CELL LYMPHOMAS TREATED WITH IFOSFAMIDE, ETOPOSIDE, EPIRUBICIN / INTERMEDIATE METHOTREXATE AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Haematologica | 2017; 102(s2) | 59

Madrid, Spain, June 22 – 25, 2017
Bone marrow failure syndromes incl. PNH - Biology

P226
IDENTIFICATION OF A NOVEL GERMINE MECOM / EVI1 VARIANT THAT RUNS IN A PEDIGREE WITH RADIOLYNAR SYNOTOSIS AND AMEGAKARYOCYTIC THROMBOCYTOPENIA AND PREDISPOSES TO ADULT ONSET MYELOID MALIGNANCY
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Background: Radiolynar 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 ectopic viral integration site 1 (EVI1) 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 radiolynar synostosis, incompletely penetrant congenital thrombocytopenia, hearing impairment due to dysplastic middle ear bones, patellar hypoplasia, and hand and foot dysmorphism. 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 known to be deleterious in the human genome. All three subjects were confirmed to be de novo carriers of a Rad51 missense variant (rad51), predicted to have an allele frequency of ≤0.1% (1000G, ESP6500, ExAC), and (iv) not listed in our in-house database of recurrent variants.

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 C2-H2-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].

P227
LOSS OF THE HOMOLOGOUS RECOMBINATION GENE RAD51 LEADS TO FANCONI ANEMIA-LIKE SYMPTOMS IN ZEBRAFISH
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Background: Fanconi anemia (FA) is a hereditary DNA repair disorder characterized by various congenital abnormalities, progressive bone marrow failure and cancer predisposition. RAD51 has recently been designated as a Fanconi anemia (FA) gene, following the discovery of two patients carrying dominant negative mutations. RAD51 is an intrinsically homologous recombination protein, necessary for strand invasion and crossing over. It has been extensively studied in prokarocytes and lower eukarocytes. However, there is a significant lack of knowledge of the role of this protein and its regulation in an in-vivo context in vertebrates due to the early embryonic lethality of murine Rad51 mutants. Aiming to fill this gap, we aim to utilize the powerful genetics and translucency of zebrafish to dissect the role of rad51 in hematopoiesis and to explore the molecular basis of Fanconi anemia pathogenesis.

Methods: Zebrafish carrying homoygous loss of function mutations in rad51

60 | haematologica | 2017; 102(s2)
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 hypoplastic 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. 

The elder daughter (age 30) TW (II.1), paternal grandfather died of “pernicious anaemia”. None of the family have anaemia with very minimal thrombocytopenia. Further investigations (Table 1) revealed lifelong macrocytosis and previous mild neutropenia (Table 1). 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 anaemia with very minimal thrombocytopenia. Further investigations (Table 1) revealed lifelong macrocytosis and previous mild neutropenia (Table 1). 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.

Summary/Conclusions: We demonstrate that zebrfish lacking functional rad51 are 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 zebrfish 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 anaemia, myelodysplastic syndrome and acute leukaemia, sometimes accompanied by non-haematological phenotypes. Here we report a likely pathogenic TERC variant associated with a haematological 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. Sampling sequencing was used to confirm the novel variant. Telomere lengths were performed at the Laboratory for Molecular Haemato-Oncology (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 anaemia 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 megakaryopoiesis 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 anaemia, 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 pedigree members with macrocytosis had a Chr3:16948268 (GRCh37) single nucleotide variant corresponding to a n.181A>C substitution in TERC (relative to transcript ENST00000602385.1), 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.

Summary/Conclusions: This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild haematological phenotype that is largely restricted to red cells. This empha-

P229

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

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Background: X-linked Dyskeratosis congenita (XDC) 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 regimens and the difficulties to find a compatible donor suggest that gene therapy may constitute a promising alternative in treating DC patients.

Summary/Conclusions: This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild haematological phenotype that is largely restricted to red cells. This empha-

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>I.1</th>
<th>I.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>130-170 g/L</td>
<td>94 g/L</td>
<td>106 g/L</td>
</tr>
<tr>
<td>Mean cell volume</td>
<td>80-100 FL</td>
<td>111 FL</td>
<td>90 FL</td>
</tr>
<tr>
<td>Mean cell haemoglobin</td>
<td>27-32 g/dL</td>
<td>33.9 g/dL</td>
<td>30.4 g/dL</td>
</tr>
<tr>
<td>Total white blood cell count</td>
<td>4-10 x 10^9/L</td>
<td>4.36 x 10^9/L</td>
<td>4.0 x 10^9/L</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2-7 x 10^9/L</td>
<td>1.67 x 10^9/L</td>
<td>2.0 x 10^9/L</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.5-4.5 x 10^9/L</td>
<td>1.67 x 10^9/L</td>
<td>1.2 x 10^9/L</td>
</tr>
<tr>
<td>Platelets</td>
<td>150-400 x 10^9/L</td>
<td>110 x 10^9/L</td>
<td>194 x 10^9/L</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate</td>
<td>&lt;15 mm/h</td>
<td>20 mm/h</td>
<td>25 mm/h</td>
</tr>
<tr>
<td>Serum iron</td>
<td>50-150 μg/dL</td>
<td>35 μg/dL</td>
<td>50 μg/dL</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>20-200 ng/mL</td>
<td>50 ng/mL</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>Serum transferrin</td>
<td>200-300 mg/L</td>
<td>150 mg/L</td>
<td>180 mg/L</td>
</tr>
</tbody>
</table>

The table shows hematological parameters for Case I.1 and I.2.
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 dyskerin in cord blood HSCs using different anti-DKC1 short hairpin RNAs (shRNA).

Methods: CD34+ cells were obtained by immunomagnetic purification from healthy umbilical cord blood samples. These cells were then pre-stimulated with 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. Intact 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 healthy umbilical cord blood HSCs allowed the generation 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 ECULIZUMAB TREATMENT: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a disease characterized by complement-mediated hemolysis (Brodaty et al., Hematology, 2008). Complement can induce the production of extracellular vesicles (EV) (Burnouf et al. Transfus Apher Sci, 2015). These EV are cell-derived vesicles whose the size-range is around 50 and 1000nm. They can expose phosphatidylserine (PS-antionic phospholipid) and tissue factor (TF), which explains their involvement in the coagulation cascade (Owens et al. Circ Res, 2011). The EV could have a role in the thrombus formation, the leading cause of death in PNH patients (Brodsky et al. Hematology, 2008; Simak et al. Br J Haematol, 2004; Hugel et al. Blood, 1999). Eculizumab, a human anti-C5 monoclonal antibody, used in the treatment of PNH seems to decrease the thrombosis frequency (relative reduction of 85% of thromboembolic event rate with the introduction of the treatment in the patients) (Hillmen et al. Blood, 2007; Kelly et al. Ther Clin Risk Manag, 2009; Weitz et al. Thromb Res, 2012; Al-Jafar et al. Hemato Rep, 2015).

Aims: The general purpose of this project is a better understanding about the role of EVs in thrombosis in the context of PNH patients under eculizumab.

Methods: We conducted a pilot prospective open label longitudinal clinical study with six PNH patients treated with eculizumab. The study was led according to 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 telomereopathies (i.e., TERT, TERC, DKC1, NOP10, NHP2, U1B,, T1EL1, TNZ, TCA1) 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 leading to TL screening were aplastic anemia (AA, n=72, 39% of cases), unexplained cytopenia (UC, n=11, 5.1% of cases), idiopathic aplastic anemia (IAA, n=0), unknown primary (UP) as the initial diagnosis in 15 out of 38 patients (39%), 4% of cases, respectively. Mutations were detected in RTEL1 (n=3), TERC (n=6), TERT (n=3) 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: Eculizumab has an impact on the amount and the procoagulant profile induced by the procoagulant PL and the EVs. The anti-thrombolytic performance of the eculizumab can be in part explained by its action on EVs.

P231

TELOMERE LENGTH SCREENING TRIGGERED BY CLINICAL SUSPICION FOR CLASSICAL AND/OR CRYPTIC DYSKERATOSIS CONGENITA – PROSPECTIVE RESULTS FROM THE AACHEN TELOMEROPATHY REGISTRY

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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 WPSAA-EU prospective 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 oftengrafted from alternative 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 on 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). Infecions remained 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).

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.
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 immunemediated attack of marrow precursors thus generating a phenotypic overlap that can impair the correct diagnosis.

Aims: 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 multiligneage (63) MF. 48 (54%) 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 PIK3CD, 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 and PIGF 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 subc cutaneous (SC) injection which can be self-administered by patients.

Aims: 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. After being suitably instructed patients are encouraged to self-inject the drug. 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 co-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 significa nt 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.
GERMLINE RARE VARIANT ASSOCIATION ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: CLL is a highly heritable cancer. Although GWAS have identified ~30 independent SNPs associated with CLL, these are estimated to account for only 10% 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 IgC. 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 study of genetic risk for myocardial infarction. We combined these heterogeneous datasets by: (i) pooling sequencing data from all cohorts together and consistently; (ii) jointly calling the variants across all cases and controls; and (iii) analyzing only ethnically matched, unrelated samples over DNA sites with sequencing coverage sufficient to achieve high-confidence genotype calls. This quality control resulted in 8,920 controls available for the association analysis. We further controlled for residual population stratification by correcting for three principal components.

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 2.6-13.1; p=5.8x10-7 and OR 3.0, 95% CI 1.3-7.5, p=0.0003, respectively). CDK1 variants were observed in 2 of 516 CLLs and 24 of 8,920 controls (1.6% vs. 0.3%, OR=5.6, 95% CI 2.6-13.1). One recurrent missense variant, CDK1 p.R59C, observed in 5 cases and 10 controls, is predicted to be possibly damaging by the PolyPhen2 prediction 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 141 controls carrying 126 rare variants (21.7% vs. 9.0%, OR=2.4, 95% CI 1.4-4.3; p=1.3x10-3). The majority of recurrent rare variants in ATM were non-synonymous missense variants, with L320T being the most enriched (2.3% cases, OR=10.1, 4.9-20.7). Subsequent validation in 149 independent CLL cases revealed a similar frequency of 2.01% (3 out of 149) of the L320T 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 two-stage replicate analysis. We identified 42 additional patients with rare ATM variants, and the significance of ATM was greatly increased (p=0.00016, OR 1.79, CI 1.49-2.15). We integrated somatic and germline sequencing data and performed an expanded joint analysis, which has been shown to improve the association. The second significant gene was ATM, in which we found a total of 112 cases carrying 52 distinct rare germline variants and 141 controls carrying 126 rare variants (21.7% vs. 9.0%, OR=2.4, 95% CI 1.4-4.3; p=1.3x10-3). The majority of recurrent rare variants in ATM were non-synonymous missense variants, with L320T being the most enriched (2.3% cases, OR=10.1, 4.9-20.7). Subsequent validation in 149 independent CLL cases revealed a similar frequency of 2.01% (3 out of 149) of the L320T 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 two-stage replicate analysis. We identified 42 additional patients with rare ATM variants, and the significance of ATM was greatly increased (p=0.00016, OR 1.79, CI 1.49-2.15). We integrated somatic and germline sequencing data and performed an expanded joint analysis, which has been shown to improve the association. The second significant gene was ATM, in which we found a total of 112 cases carrying 52 distinct rare germline variants and 141 controls carrying 126 rare variants (21.7% vs. 9.0%, OR=2.4, 95% CI 1.4-4.3; p=1.3x10-3). The majority of recurrent rare variants in ATM were non-synonymous missense variants, with L320T being the most enriched (2.3% cases, OR=10.1, 4.9-20.7). Subsequent validation in 149 independent CLL cases revealed a similar frequency of 2.01% (3 out of 149) of the L320T 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 two-stage replicate analysis. We identified 42 additional patients with rare ATM variants, and the significance of ATM was greatly increased (p=0.00016, OR 1.79, CI 1.49-2.15). We integrated somatic and germline sequencing data and found that patients with rare germline variants in ATM were more likely to harbor an additional ATM somatic deletion (p=9.1x10-4). Furthermore, 80% of patients with both a rare germline variant in ATM and a somatic 11q deletion lost the wild-type ATM allele during deletion (p=0.012), suggesting that the germline variants behave as tumor suppressor alleles.

Summary/Conclusions: To our knowledge this analysis represents the first germline association analysis based on exome sequencing data in CLL, and our results implicate rare germline variation in ATM in CLL predisposition.
17p13 deletions were assessed by FISH (MetaSystems). More than a half of the cohort (57%) was also analyzed using ultra-deep NGS for TP53 exons 2-11. Genome-wide analysis was performed on CytoScanHD arrays (Affymetrix) and correlated to conventional cytogenetics (CpG/LSI-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 variants heterozygous for TP53mut to explore the potential presence of 17p cn-LOH. In 42% (18/43) of the cases, cn-LOH in 17p 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 with a CNLOH status and would have escaped detection by Sanger sequencing. Therefore ten of the 26 patients were identified in whom the mutation analysis was performed with a sensitivity up to 1% mutant allele frequency. For the determination of the 17p status we have used a high resolution CytoScan HD array which allows a genome-wide detection of copy number alterations (CNAs), down to 100 kb in size, and regions of copy neutral loss of heterozygosity (CNLOH). In addition the presence of TP53 mutations was evaluated with high sensitivity using next generation sequencing approaches.

Methods: We have studied bone marrow or peripheral blood samples of 179 CLL patients using oligo/SNP-based array which allows a genome-wide detection of copy number alterations (CNAs), down to 100 kb in size, and regions of copy neutral loss of heterozygosity (CNLOH). In addition, 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 CNAs detected by FISH at progression: two pts had del(13q) and del(11q) and one pt developed tetrasomy 12. In the absence of disease progression, the only CCE detected was emergence of small sub-clones. Conventional karyotyping was performed in 10/37 patients who progressed both pre-treatment and at progression. In 4 pts, CCE was identified at progression, suggestive of tetraploidy. 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 was clonally unrelated. Median FISH%del(17p) pre-treatment in those with del(17p) was 72%; only 1 pt had <50% del(17p) pre-treatment. All these pts had persistence of del(17p), at progression, without significant changes in allelic frequency. Two pts with del(17p) pre-treatment had additional abnormalities detected by FISH at progression: sub-clonal biallelic del(13q) was seen in two pts, one of whom also developed tetrasomy 12. In the absence of disease progression, the only CCE detected was emergence of small sub-clones with biallelic del(13q) in two patients who initially had monoallelic del(13q). Notably, in responding pts, there was no expansion of high-risk sub-clones. Conventional karyotyping was performed in 10/37 patients who progressed both pre-treatment and at progression. 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.
Pairwise association showed statistically significant co-occurrence between tri(17p) and mutations in KRAS/BCOR (both \(p<0.05\)), NOTCH1 mutation and ZMYM3 (\(p=0.01\)), SPEP (\(p<0.05\)) mutation, 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 mutation in SF3B1 (\(p=0.026\)), MGA (\(p=0.035\)), DDX3X (\(p=0.001\)), 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). In R/R cohort, complex karyotype, del(17p) and mutations in SF3B1 and TP53 were associated in ibrutinib treated patients. DDX3X 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.

**P242**

HIGH THROUGHPUT IMMUNOPROFILING OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS ASSIGNED TO STEREOTYPED SUBSET #4: NOVEL INSIGHTS INTO THE DEPTH, DIVERSITY AND TEMPORAL DYNAMICS OF CLONAL EVOLUTION

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Background: Chronic lymphocytic leukemia (CLL) clones assigned to stereotyped subset #4 are characterized clinically by a young age at diagnosis, an indolent disease course, and molecularly by B-cell receptor immunoglobulins (BcR Ig) that exhibit distinctive immunogenetic features. More specifically, they are IgG-switched, composed of heavy and light chains encoded by the IGHV4-34 and IGKV2-30 genes, respectively, and their heavy chain complementarity determining region 3 (V(H)CDR3) is long and enriched in positively charged residues, reminiscent of pathogenic anti-DNA antibodies. In addition, both the VH and VK domains of subset #4 demonstrate a high impact of somatic hypermutation (SHM), highly indicative of an (auto)antigen selection.

Aims: To obtain comprehensive insights into the ontogeny and evolution of CLL subset #4 using next-generation sequencing (NGS) for in-depth immunoprofiling of the clonotypic BcR Ig genes, particularly focusing on analyzing intraclonal diversification (ID) within the IDG gene sequences.

Methods: Peripheral blood samples were collected at multiple time-points over a 10-year period from 6 CLL subset #4 patients. The clonotypic IGVH-IGHD-IGHJ and IGKV-IGKJ rearrangements were amplified by PCR using cDNA and sequenced on the MiSeq (Illumina). Our experimental design involved paired-end sequencing, thus allowing sequencing of the CDR3 twice/read, so as to increase the accuracy of results. To maintain stringency, raw NGS reads were subjected to purpose-built, bioinformatics algorithms, which filtered prepared: (i) length and quality filtering of raw quality filtered reads; (ii) filtering of filtered-in paired reads via local alignment; and, (iii) length and quality filtering of stitched sequences. No base calls of Q-score<30 were allowed in the 75 nucleotide stretch preceding the GXG motif, further increasing CDR3 sequencing reliability. Data was then analyzed using the IMGT/HighV-QUEST database and clonotype computation was performed using an in-house bioinformatics pipeline.

Results: Overall, 48 samples were analyzed, producing 12,386,554 and 4,506,464 total reads for heavy and light chain, respectively. In addition to filtering out poor quality, incomplete, out-of-frame and unproductive rearrangements, we have imposed specific parameters (\(k<0.5\), \(q<0.01\)), usage of subset #4-specific V- and J-genes, CDR3 length and landmark residues. Applying these strict criteria resulted in 84.1% (median 401,133 reads/sample) and 90.3% (median 141,549.5 reads/sample) of the total sequences obtained for the heavy and light chain, respectively, passing filters. Clonotype computation was solely based on the filtered reads, and revealed a median of 1332.5 clonotypes/sample (range: 879-3432) for the heavy chains while a median of 202.5 clonotypes/sample (range: 125-395) was evidenced for the light chains. Overall, our longitudinal analysis revealed: (i) a hierarchical pattern of subclonal evolution showing which SHMs were more likely to emerge and/or positively selected; (ii) several distinct clusters of subcloned sequences which at later time-points had often disappeared and hence been selected against; and, (iii) that despite the high intensity of ID, certain residues remained essentially unaltered alluding to strong functional constraints.

Summary/Conclusions: Detailed molecular immunoprofiling by NGS afforded the possibility to gain novel insights into the pathogenesis of CLL subset #4, thus providing conclusive evidence that these patients continue to acquire SHMs within their Ig genes; an observation best explained by a clear role for antigen selection in clonal evolution.
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 program 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 pathogenesis.

Methods: Clonal B cell specimens from 122 CLL patients were subjected to DNA methylation profiling using Illumina 450k arrays. 17 CLL 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 normal 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 the 122 CLL patients into three major epigenetic groups corresponding to IGHV unmutated and mutated CLL, respectively; and iii) a naïve like CLL (n-CLL), ii) good-prognostic, memory like CLL (m-CLL), broadly grouped based on their epigenetic classification, revealed that subset #1 clustered with n-CLL, subset #4 with m-CLL, while subset #2 clustered separately from both groups.

Summary/Conclusions: Here, we demonstrate that 5hmC loss in CLL contributes to a disease specific epigenotype as described earlier. First evidences indicate that alterations of an interaction between the EBF1 and TET2 are mechanistically involved in insufficient hydroxymethylation and consequently failed DNA hypomethylation.
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 IcICLLe Extension Study expands on the IcICLLe trial (ISRCTN12696354) to examine the efficacy and safety of the combination treatment of obinutuzumab and ibrutinib.

Aims: The IcICLLe trial was a single-arm, multicentre feasibility study that recruited 40 participants with CLL requiring treatment to receive continuous ibrutinib therapy from day 0 and 6 cycles of obinutuzumab treatment. There were 2 separate reports of grade 2 infusion related reactions, 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-naïve cases peripheral blood (PB) CLL cell 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 8-85%; P=0.003). Pretreatment BM aspirate matched-pairs were ranked. 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 ≥1 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.

Results: Among the 32 patients with aggressive CLL, we found that 9 (28%) patients who had an IgL3V21 rearrangement, but only 1 patient carried the heavy chain IgHV3-21: IgL3V21-21 patients had a median TFS of 17 months compared to 44 months in patients with another light chain (P=0.0270). Similarly, IgL3V21 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 IgL3V21 light chain and 10 (4%) an IgHV3-21 (of which 8/10 also carried the light chain IgL3V21 rearrangement). Patients with IgL3V21 had a median TFS/OS of 29/183 months compared to patients without IgL3V21 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 IgL3V21 light chain but only 1 (1%) had a heavy chain IgHV3-21. In this cohort, IgL3V21 patients had a median TFS of 21 months not statistically different from IgHV3-21 patients (28 months) while IgHV3-21 patients who 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 IgL3V21 with the IgHV mutational status: patients with either IgL3V21 or IgHV3-21 (with a M or UM IgHV) displayed a prognosis similar to UM patients: median TFS was 129, 48, 36, 24, 23 months for M, IgL3V21/M (P=0.0005), UM (P<0.0001), IgL3V21/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, IgL3V21/M (P<0.0001), UM (P<0.0001), IgL3V21/UM (P<0.0001) and IgHV3-21 (P=0.0021) patients, respectively (Figure 1B). If all IgL3V21 (n=48) were considered independently of their heavy chain, IgL3V21 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).

Background: The immunoglobulin heavy-chain gene (IgHV) mutational status is currently considered the gold standard of prognostication 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 IgL3V21/IgHV3-21 who have poor prognosis irrespectively of the IgHV mutational status. Interestingly, IgL3V21 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: Based 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 IgL3V21 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 IgL3V21 rearrangement, but only 1 patient carried the heavy chain IgHV3-21: IgL3V21-21 patients had a median TFS of 17 months compared to 44 months in patients with another light chain (P=0.0270). Similarly, IgL3V21 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 IgL3V21 light chain and 10 (4%) an IgHV3-21 (of which 8/10 also carried the light chain IgL3V21 rearrangement). Patients with IgL3V21 had a median TFS/OS of 29/183 months compared to patients without IgL3V21 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 IgL3V21 light chain but only 1 (1%) had a heavy chain IgHV3-21. In this cohort, IgL3V21 patients had a median TFS of 21 months not statistically different from IgHV3-21 patients (28 months) while IgHV3-21 patients who 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 IgL3V21 with the IgHV mutational status: patients with either IgL3V21 or IgHV3-21 (with a M or UM IgHV) displayed a prognosis similar to UM patients: median TFS was 129, 48, 36, 24, 23 months for M, IgL3V21/M (P=0.0005), UM (P<0.0001), IgL3V21/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, IgL3V21/M (P<0.0001), UM (P<0.0001), IgL3V21/UM (P<0.0001) and IgHV3-21 (P=0.0021) patients, respectively (Figure 1B). If all IgL3V21 (n=48) were considered independently of their heavy chain, IgL3V21 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 IgTN-21 in CLL: the presence of an IgL3V21 light chain confers a poor prognosis similar to UM patient irrespectively of concurrent expression of IgHV3-21 heavy chain or IgHV mutational status.
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 remained in follow-up and remained on study as allowed off therapy could re-initiate venetoclax and rituximab.

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.

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 using ≥4-color flow cytometry (minimum sensitivity: 0.01%). Patients who achieved MRD-negativity in the bone marrow who remain on study 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.

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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 TP53). Regarding NOTCH1, ofatumumab was only beneficial in NOTCH1mut but not in NOTCH1mut patients (HR 0.64, p=0.01 and HR 0.86, p=0.367) (Figure 1).

Summary/Conclusions: In the COMPLEMENT-2 trial evaluating FCO against FC in relapsed/refractory CLL patients, we found TP53mut and XPO1mut but not SF3B1mut or NOTCH1mut as independent prognostic factors for PFS. Notably, the addition of ofatumumab to chemotherapy did not result in a benefit among NOTCH1mut but not among NOTCH1mut 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 UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA

A. Dinmohamed1,2,3,* O. Visser4, W. Posthuma5, R. Raymakers6, J. Doorduijn3

signals were observed.

to study treatment or CLL by investigators.

No unexpected safety

Incidence of Gr 3–4 infections was 11.8%. Incidence of tumor

cytopenia (26.5%). Incidence of Gr 3–4 infections was 11.8%. Incidence of tumor

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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 revolution, 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 1,000,000 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 years; 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 2001-2014). 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 of pts who did not receive any 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 85% (77% - 92%; P<.036; 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.

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 age ≥60, but this was not statistically significant for pts age ≥70. 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.
Background: CCL-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 ECOG performance score of 0 or 1. Complete information on age, Binet stage, beta-2-microglobulin, 17p deletion / 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. Comorbidities were most frequently captured under the following range (0-22); 81% of the patients had a total CIRS score of at least 1 and 28% (40%) high risk, 121 (7%) very high risk. The median total CIRS score was 3 (range 0-22); 81% of the patients had a total CIRS score of at least 1 and 28% of greater than 6. Comorbidities were most frequently captured under the following categories: cardiac, blood pressure, respiratory, musculoskeletal, or endocrine/metabolic. A severity score of >2 and >3 in at least one CIRS organ category was present in 46% and 11% of the patients, respectively. There was a positive correlation between total CIRS score and age (r=0.5, p<0.001) as well as ECOG performance score (r=0.4, p<0.001) and an inverse association between total CIRS score and creatinine clearance (r=-0.3, p<0.001). In uni- and multivariable analysis, increased total CIRS score was associated with shorter overall survival (OS); with poorer OS determined by severity rather than numbers of comorbidities (log-rank: p<0.001, Figure 1A and 1B). In multivariable analysis, total CIRS score was an independent risk factor for OS when used as continuous or categorical variable together with age, gender, Binet stage, ECOG performance score, thymidine kinase, beta-2-microglobulin, IGHV, and 17p deletion (adjusted for treatment intensity). Total CIRS score also remained an independent risk factor for OS when added to the CLL-IPI. Weight of CIRS was highest in the CLL-L and lower in the CLL10 and CLL11 trials as expressed by the hazard ratios (Figure 1C). There was no significant association between total CIRS score and progression-free survival or time-to-next treatment. However, increased total 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 above and beyond the CLL-IPI. Systematic comorbidity assessment (e.g. by CIRS) in addition to the CLL-IPI therefore appears reasonable when estimating overall prognosis and deciding treatment in 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 C1–2–6) with FC (fludarabine 250mg/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) or 30 mo (G-B). At last follow-up in follow-up; G-FC (n=18: 2 lost to follow-up) and G-B (n=19: 1 event of progressive disease 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), both Gr3). Gr4 leukopenia/neutropenia, small cell lung cancer and Gr4 pneumoehorax, 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 <0.07x109/L). Within 6–12 mo of follow-up, very few pts had recovered (<1 Gr2) except one in the G-B arm. Disease status at 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&l&l;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 patient and disease (including CLL-IPI), and transplant characteristics predicted for 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: 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 median (25%–75% interquartile range) and categorized 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%.

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

Aims: The expression levels of efflux and influx channel transcript levels were investigated by a multivariate Cox’s regression model. Relapse has been defined as a two-time higher risk of relapse in multivariate analysis. Further prospective validation is warranted.

Results: In our cohort, 132 chronic phase CML patients discontinued TKI treatment. The predictive significance of the efflux transporter correlated with a two-time higher risk of relapse in multivariate analysis. Further prospective validation is warranted.

Summary/Conclusions: Here we investigated for the first time the impact of pharmacokinetics in the context of a CML discontinuation trial. ABCG2 but not OCT1 and ABCB1 (MDR1) predicted treatment-free remission after TKI discontinuation. High expression of the ABCG2 efflux transporter correlated with a two-time higher risk of relapse in multivariate analysis. Further prospective validation is warranted.
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 LEUKEMIA IN CHRONIC PHASE: ENESTOP 96-WK UPDATE


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. The primary analysis, 57.9% of pts (73/126) who stopped treatment remained in TFR (no confirmed loss of MR4 [BCR-ABL1 IS ≤0.01%], and no treatment reinitiation) at 96 wk. All pts provided informed consent. Enrolled pts continued NIL at 38.4±7.1 mg/l. In contrast, second line NILO showed a trend for increase 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 is 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.

Methods: Differentiating 3T3-F442A, a mouse adipocyte line, were differentiated with clinically relevant concentrations of NILO (1-20µM) and IM (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 was assessed by semiquantitative real-time PCR, and adipokine secretion using an in vitro adipocyte model; ii) To utilise the in vitro model to explore potential therapeutic strategies to reverse NIL-mediated effects, and iii) To validate the in vitro results in a pilot patient cohort.

Results: NILO and IMA resulted in significant downregulation of GLUT4 mRNA (NILO, 54%; IMA, 55%; p<0.01 in comparison to vehicle control, 79.2ng/ml). Co-incubation with telmisartan resulted in significant reversal of NILO-mediated effects on GLUT4 expression and adipokine secretion.

Conclusions: The results demonstrate the durability of TFR after stopping NIL in pts who achieved a sustained deep MR after switching from IM to NIL.
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 TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Background: A BCR-ABL1 transcript level at 3 months after the initiation of imatinib has shown predictive value in the clinical outcomes of patients with chronic myeloid leukemia in chronic phase (CP-CML). The levels obtained earlier than 3 months may also have a similar prognostic significance.

Aims: To assess the prognostic value of the BCR-ABL1 transcript levels at baseline, and 1 and 3 months after the initiation of a tyrosine kinase inhibitor (TKI) in predicting the major molecular response (MMR) achievement by 12 months, and to compare the patterns of molecular response (MR) to a TKI therapy between good and poor responders using a nonlinear model.

Methods: The clinical data were collected from the 178 patients with newly diagnosed CP-CML who were treated with a TKI at Seoul St. Mary’s Hospital. BCR-ABL1 transcript levels were obtained at baseline, and 1, 3, and 6 months after the initiation of a TKI. The levels were reported as the percent ratio relative to the control gene ABL1 in accordance with the International Scale (BCR-ABL1/ABL1[%)]. A confirmed MMR was defined as a BCR-ABL1/ABL1[≤0.1%] on two consecutive occasions. The predictability of the levels at baseline, and 1 and 3 months post TKI therapy for the achievement of a confirmed MMR by 12 months was evaluated using a logistic regression method with a receiver operating characteristic (ROC) analysis. The areas under the ROC curve (AUCs) were calculated to quantify the predictability. In addition, the patterns of molecular responses over time were described by a nonlinear model with the model-derived parameters between the patients who achieved a confirmed MMR by 12 months (“good responders”) and who did not achieve the MMR (“poor responders”).

Results: Of 178 patients, 67 achieved a confirmed MMR by 12 months but 111 did not. At baseline, the transcript level was not useful to predict the achievement of a confirmed MMR by 12 months. At 1 month post therapy, the levels measured at 1 month significantly (p < 0.0001) predicted the MMR with an AUC of 0.77. The patients with the level of 38% or less at 1 month had a better chance to achieve the MMR. By 3 months post therapy, the transcript level measured at 3 months (p < 0.0001) accurately predicted the MMR with the AUC of 0.83. The patients with the level of 0.48% or less at 3 months had a better chance to achieve the MMR. A nonlinear sigmoid model was used to fit the transcript data from 149 patients as follows: MR=MR0 [1 – tγ/ (t50γ+tγ)]; where MR0 is the predicted molecular response at baseline; t, time post TKI initiation; γ, slope factor; t50, time required to achieve 50% reduction in MR. Statistically significant differences were observed between the good and poor responders in the median values for the model-derived parameters of MR0 (73.3% vs 82.2%; p=0.003), γ (4.98 vs 3.32; p < 0.0001) and t50 (0.952 month vs 1.12 month; p=0.05).

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: Risk scores in chronic myeloid leukemia (CML) use baseline characteristics of CML patients in chronic phase to predict outcome and can be used to make decisions regarding first line TKI choice and monitoring frequencies. Until recently, risk stratification of CML patients was based on scores developed in the pre-imatinib era (Sokal and Hasford risk score) with overall survival as the end point of interest. After the introduction of imatinib, the EUTOS score was established to predict the risk of relapse in CML in patients treated with first line imatinib.

Aims: The primary objective of this study was to perform a validation of the ELTS score in an independent cohort of “real-world” population-based CML patients.

Methods: Data from chronic phase CML patients were derived from the PHAROS-CML population based registry and Hemobase. Patients were stratified into a low, intermediate and high risk group according to the ELTS score. Data on “death due to CML” were provided by the Netherlands comprehensive cancer organization (IKNL) in combination with details from the patient records and a competing risk analysis was performed, to take death due to other causes into account.

Results: In total 349 patients were eligible for analysis; 273 patients (78%) were treated with first line imatinib and 76 patients (22%) were treated with a first line second generation TKI (2GTKI). Sokal, Hasford and EUTOS risk scores all did not predict differences in risk of “death due to CML.” The ELTS score identified 163 patients as low risk (47%), 127 patients as intermediate risk (36%) and 59 patients as high risk (17%) at diagnosis. The 5 year cumulative incidence of “death due to CML” was indeed significantly higher in the high risk group (11%) compared to both the intermediate risk group (2%, p<0.02) and the low risk group (1%, p<0.001). Between the intermediate and low risk group no statistically significant difference in risk of dying from CML was observed. A subgroup analysis of only imatinib treated patients showed similar results.

Summary/Conclusions: In the current study based on a “real-world” population-based CML patient cohort, we were able to validate the predictive value of ELTS high risk stratification for “death due to CML” in the current TKI era. Therefore, the ELTS score should be preferred over Sokal, Hasford and EUTOS scores in clinical practice.

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 Haematology, 2015; 2 (12):e528-35) and demonstrated that dasatinib could be safely discontinued in patients with a DMR for at least 12 months, and that longer follow-up results would clinically 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 ROC-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. ROC-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 ROC-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 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 reconfirmed 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 ex 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 hypoxia. 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 (Tu 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 mimics 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 and 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 during engraftment we explored: i) scaffolds material composition (bioactive glass to phosphate (BCP) 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 homogenous mixture of human hematopoietic cells CD31-positive and CD44, CD146, LEPR and nestin-positive stromal niche cells. Comparison of the composition and the shapes of the scaffolds indicated superior of TCP and tube-shaped scaffolds in supporting the formation of vessels. Engraftment of BM-derived CD34+ cells in the CB-EPC embedded huBM-sc resulted in increased multilineage hematopoietic engraftment, as compared to huBM-sc without CB-EPCs. Moreover, we observed that incorporation of CB-EPCs provides faster kinetics of engraftment of both patient-derived BM and AML cells, and proved to be essential for the engraftment of blast cells from myelofibrosis patients.

Summary/Conclusions: Thus, with the addition of human CB-EPCs and BM stromal cells, our scaffold systems now simulate both endosteal 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 through blood vessel 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, playing fundamental regulatory roles in hematopoietic development. Recent evidence suggests that tissue regions around BM venous microvessels (termed sinusoids), which are enriched for mesenchymal CXCL12-12-abundant reticular cells (CARCs), 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, large LAIR1 insertions at the V-D junction were described as a novel mechanism to generate antibodies against RIFIN antigens on infected erythrocytes (Tan et al., Nature 2016). These templated insertions potentially add a new 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 exploited 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=10−10−0). These sequences represented all VDJ sequences with a CDR3 region >150 bp. Exonic sequences from RPLP0, ZNF316, and an inverted IGHV-IGHD sequence were identified as insertions in unmutated IgM VDJ transcripts. The LAIR1 exon described by Tan et al. and an intergenic region adjacent to IGHD3-22 were identified as insertions in IgG VDJ transcripts. One IgA VDJ contained two intergenic sequences positioned close to IGHV4-37 in a homozygous chromosome 22. Somatic hypermutation correlated strongly between the IGHV segment and the templated insertions (r=0.9944; p<0.0001). All templated insertions harboured cryptic RSS sites at their termini. All three IgG VDJ carrying templated insertions and the IgG rearrangement with a templated insertion gave rise to detectable surface immunoglobulin after coexpression with at least one light chain in the panel. The IgG VDJ carrying the LAIR1 templated insertion produced no detectable surface immunoglobulin. In contrast, the VDJ sequences carrying LAIR1 templated insertions as described by Tan et al. could be expressed with the majority of the light chains. The IgA rearrangement remained to be tested in this system.

**Summary/Conclusions:** Templated insertions represent a novel antibody diversification mechanism. Their presence in naïve B-cells, their exclusive positioning in VDJ junctions, and the universal presence of cryptic RSS sites point to primary VDJ recombination or secondary V gene editing as the generating mechanism. Certain loci (e.g. LAIR1) and in some individuals appear to have increased susceptibility. The available data suggest RAG to be involved in these insertions. We propose that templated insertions represent inserted small sequences from aberrant rearranged chromosomal sequences with cryptic RSS sites.

**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 diverse 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-3−/− and caspase-7 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 cytotoxic 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 inhibitor molecule, including zVAD-fmk and the clinically developed IDN6556. This effect was associated with a change in the phenotype 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 therapeutic strategy to modulate macrophage polarization with diverse applications including cancer treatment.

**P269**

**MULTIPLE MYELOMA-POLARIZED M2C MACROPHAGES PROMOTE A TUMOR-SUPPORTIVE OSTEOLOGY MICROENVIRONMENT VIA CXCL13**

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**Background:** Previous studies including our work revealed a role of MM-edited M2-like macrophages (MΦ) in MM survival and drug resistance. However, the mechanism by which neoplastic plasma cells shape BM microenvironment and affect MΦ polarization is still poorly defined.

**Aims:** To investigate tumour macrophage polarization in the myeloid compartment of the BM niche that are initiated by MM cells.

**Methods:** We utilized our in vivo xenograft model of BM disseminated human myeloma. The CRISPR/cas9 technology was used to knockdown CXCL13 expression in MM cell lines.

**Results:** BM analysis of mice inoculated with human CXCR4-expressing RPMI8226 cells revealed a significant increase in M2c MΦ in comparison to non-injected controls (p<0.01). Characterization of MM-associated changes in the BM milieu revealed myeloma chemotractant CXCL13 being one of the most profoundly increased factors upon MM development. Elevated CXCL13 was also detected in blood of MM-bearing animals comparing to healthy controls. IHC staining identified myeloid cells as the main source of increased murine CXCL13, both in BM and blood, suggesting the possible utilization of CXCL13 levels as surrogate marker of anti-MM treatments response.

**Conclusion:**CXCL13 expression by MΦ is known to represent specific inflammatory MΦ type, we acquired murine xenograft system for the observation of tumour-induced polarization of additional M2c markers, such as MERTK and MR1 in the BM occupied with MM in strong correlation with CXCL13 expression (p<0.001 R2=0.65). In vitro studies confirmed the ability of MM cell lines (n=6) to induce CXCL13 and current expression of M2c markers (MERTK, CD206, CD163) in co-cultured myeloma and MΦ cells. The anti-inflammatory and anti-MM effects of the treatment with anti-CXCL13 zomib and panobinostat resulted in corresponding correlated decrease in murine CXCL13, both in BM and blood, suggesting the possible utilization of CXCL13 levels as surrogate marker of anti-MM treatments response.

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clastogenic assays were used to elucidate the down-stream effects of the elevated CXCL13. Recombinant CXCL13 opsonized as in human 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 cell lines 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. Reduced 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).

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.

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 leukemia initiation. Therefore, understanding the human fetal B cell hierarchy, especially the differences between PreProB and ProB progenitors may be key for understanding the origins of childhood leukemia.

Aims: To define the fetal B cell developmental hierarchy in the second trimester FBM, with a view to establishing the fetal B cell hierarchy.

Methods: The characteristics of the haematopoietic stem cell (HSC), lymphoid-myeloid multipotent progenitor (LMPMP), early lymphoid progenitor (ELP) and committed B-progenitor compartments of FBM samples were analysed by multiparameter flow cytometry. Differentiation and clonogenic assays, transcriptome analysis and single cell RQ-PCR were performed on a total of 633 endothelial precursors and 545 fetal liver hematopoietic precursors containing the indicated window of hematopoietic precursors specified during the indicated window of hematopoiesis. An alternative approach independent of embryo disruption or transplantation would more accurately reflect the true dynamics of hematopoiesis emerging during fetal development.

Aims: To determine the frequency of emerging HSCs and their progenitors throughout mammalian ontogeny.

Methods: Here, we employed the Confetti allele, in which a cassette targeted to the ROSE26 locus randomly and permanently marks cellular progeny with green fluorescent protein. We tested this formula in vitro by plating limiting dilution replicates of immortalized Confetti fibroblasts and assessing the resulting 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: ROSE26[Confetti+] Fik(-/-Cr) (mesodermal precursors, E7), ROSE26[Confetti+] Vc-adherin(+/-Cr) (mesodermal endothelial precursors, E8.5-E10.5), and ROSE26[Confetti+] Vav1(+/-Cr) (hematopoietic progenitors E11.5-E14.5). This correlation was used to estimate the number of hematopoietic precursors emerging during each stage of development.

development. We thereby report for the first time that the clonal origin of blood is much more complex than previously thought, with hundreds of precursors contributing to the establishment of the mammalian blood system at multiple stages of ontogeny.

P272

A20 RESTAINS THYMIC REGULATORY T CELL DEVELOPMENT

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Background: Maintaining immune tolerance requires the production of Foxp3 expressing regulatory (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 A20, 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 A20fl/fl CD4Cre 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 bonemarrow 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 allogeogenic hematopoietic stem cell transplantation with WT BM+T cells vs WT vs A20-deficient Treg cells to analyze whether A20-deficient T reg 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−/− Treg cells efficiently suppressed effector T cell mediated graft-versus-host disease after allogeogenic 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/EBPγ 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 immuno- logical 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 C/EBPγ, as a direct C/EBPα target gene. However, the role of C/EBPγ in mast cells remains so far elusive.

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

Methods: In order to determine the role of C/EBPγ 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. To elucidate whether C/EBPγ plays role in mast cell response to bacterial infection, we challenged these mice intraperitoneally with lipopolysaccharide (LPS). We used intraperitoneal injection of distilled water to eradicate peritoneal mast cells and then monitored repopulation of peritoneum over time. To further explore the role of C/EBPγ in mast cells in vivo, we established bone marrow derived mast cells (BMMCs) and determined their growth (cell numbers), morphology (toluidine blue staining), and transcription factors expression (RT-PCR) at different time points. Degranulation potential of BMMCs was specified by measuring the percentage of b-glucuronidase released to the supernatant upon anti-TNP IgE sensitization and TNP-BSA activation. To investigate the effects of absence of Cebpg during mast cell migration, we employed transwell migration assays.

Results: We verified efficient ablation of Cebpg on mRNA and protein level in bone marrow and spleen of 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 exhibit increased number of peritoneal mast cells after LPS stimulation in comparison to WT control littermates. Surprisingly, mice lacking Cebpg presented defective peritoneal mast cell repopulation. Since mast cells are scarce and difficult to isolate from in vivo models, we employed BMMCs to investigate the effects of Cebpg ablation in mast cell development and function. We observed that bone marrow from Cebpg KO mice generated reduced number of BMMCs 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 FcεRI-mediated activation. Further, BMMCs exhibit increased expression of C/EBPα in the absence of C/EBPγ.

Summary/Conclusions: In summary, we revealed C/EBPγ as 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-PREDOMINANT HODGKIN LYMPHOMA TREATED WITHIN THE RANDOMIZED HD7-HD15 TRIALS: AN ANALYSIS FROM THE GERMAN HODGKIN STUDY GROUP

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Background: Nodular 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: HD6, 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 refractory disease or relapsed during the course of follow-up (primary disease progression: 84 patients; early relapse: 6 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 89.9% (95% CI: 87.3–92.4); the median survival was 161 months (19–139). The 5-year OS rate was 89% (95% CI: 82–92); the median OS was 197 months (12–249).

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 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.

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: Hodgkin lymphoma (NHL) occurred in 48/471 patients (10%) (solid tumor: 25 patients; leukemia: 7 patients; NHL: 13 patients; unspecified malignancy: 4 patients). The 5-year OS rate was 89% (95% CI: 82–92); the median OS was 197 months (12–249).

Methods: Between June 2001 and December 2009, we analyzed 306 patients – either newly diagnosed or relapsed during the course of follow-up (primary disease progression: 84 patients; early relapse: 6 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). The 5-year OS rate was 89% (95% CI: 82–92); the median OS was 197 months (12–249).

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P277

IMPACT ON SURVIVAL OF EARLY DETECTION OF RECURRENCE IN THE FOLLOW-UP OF HIGH RISK HODGKIN LYMPHOMA IN FIRST COMPLETE REMISSION

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Background: Despite the high complete response (CR) rate to anthracycline-including first-line therapy, approximately one-third of patients with advanced-stage Hodgkin lymphoma (HL) relapses. Many relapses (30–50%) are clinically asymptomatic, without any physical and/or laboratory signs. For patients at high-risk of relapse, a close monitoring, based on imaging procedures is justified if an early detection of recurrence would allow a timely administration of salvage therapy and a survival improvement.

Aims: The purpose of this study was to evaluate the response rate to salvage therapy of relapsed HL by comparing patients who received surveillance with conventional clinical assessments versus patients who received surveillance with imaging procedures. The primary end-point was to assess the rate of CR to salvage therapy at first relapse (confirmed by FDG PET/TC performed before 1st line chemotherapy in 93% of patients). Secondary endpoints were: the overall rate of recurrence after first salvage therapy, the OS at 3 years, the CR rate at 3 years, and the OS at 5 years.

Methods: We collected data retrospectively from 8 hospitals in the East of England, from a 10-year period from a referral population of 2.64 million (incidence: 0.95 cases per 100,000). Six of the 8 centres introduced escB vs. ABVD (PFS 95% vs 82%; HR 4.3 (95% CI:1.97–9.7), p=0.0261, but there was no difference in OS (5-year OS 97% vs 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 95% vs 87%; HR 3.0 (95% CI:1.43–6.89), p=0.00001 vs 82%; HR 3.0 (95% CI:1.43–6.89), p=0.00325). 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 infertility is an important consideration for escB patients. In our patient population, of the 20 pre-menopausal women treated with escB, 11 of the 14 (78.6%) aged <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). Only 1 of the 6 (16.7%) pre-menopausal women treated with ABVD reached >30 years at diagnosis regained 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, 29 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 SNAP-US plus CXR to monitor patients with advanced stage HL. We show that SNAP-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.

**P278**

**LATER LINE DRUG TREATMENT PATTERNS OF CLASSICAL HODGKIN’S LYMPHOMA PATIENTS IN CANADA, FRANCE, GERMANY AND THE UNITED KINGDOM.**

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**Aim:** To understand the natural history and treatment patterns of patients with advanced stage classical Hodgkin’s lymphoma (cHL). Real-world data indicates an unmet medical need for new treatment strategies.

**Methods:** 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 IV (n=385) disease (p=0.99). Treatment modalities were similar across the markets; Canada (34%), France (35%), Germany (30%) and the UK (44%) (p=0.10).

**Results:** The most commonly prescribed 3rd line 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 treatments were DHAP (8%), BEAM (7%) and bendamustine (7%). 4% of 3rd line patients received a PD-1 inhibitor. 3rd line BV patients the majority received ABVD (69%) or BEACOPP (19%) at 1st line. 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). 3rd line patients treated with a PD-1 inhibitor 7% had been previously treated with BV. Data for 453 cHL 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).

**Summary/Conclusions:** Our large data cohort shows the presence of B symptoms in a state-wide cancer registry for cHL patients treated with chemotherapy alone vs patients treated with both chemotherapy and radiation.
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.

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 allo-SCT or treatment with IC-inhibitors (data not shown). 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=3.45, (95% CI, 3.35-3.55), p=0.001] were associated with decreased OS, while 3) Response after 1st 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). The endpoint was the overall response rate (ORR); secondary endpoints were overall survival (OS), PFS, and safety. The response was assessed by positron-emission tomography/computed tomography or CT. Early radiological evaluation 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.

NIVOLUMAB FOR RELapsed OR REFRACTORY HODGKIN LYMPHOMA: EXPERIENCE IN TURKEY


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Background: The programmed death-1 (PD-1) inhibitors have been approved by FDA for patients who relapse following autologous stem cell transplantation and brentuximab vedotin (BV) therapy.

Aims: This retrospective multicenter study aimed to provide information about the efficacy and safety of nivolumab in the “real-life” setting in Turkey.

Methods: 23 centers from Turkey participated in this study. Eligible patients were required those treated with at least 1 course of nivolumab and with available radiological response evaluation. The decision about inclusion of patients with an ICC decision was made by the attending physician. Patients received nivolumab via a named-patient program, and there was no restriction for BV-and/or transplantation-naive cases. Nivolumab was administered at a dose of 3 mg/kg iv infusion over 60 min q2wk in outpatient setting until death of any cause, unacceptable toxicity, withdrawal of consent, or primary physician’s decision. The study was approved by the local ethical committee. The primary endpoint was the overall response rate (ORR); secondary endpoints were overall survival (OS), PFS, and safety. The response was assessed by positron-emission tomography/computed tomography or CT. Early radiological evaluation

Figure 1.
Results: Between 06/2015-11/2016, 87 patients were enrolled in a name-based program in Turkey. Two, 19, and 5 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 66% 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 0.55-0.82) at 12-months. Median OS was not reached, while, according to the late radiative responses, 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 post-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).

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 under-going 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 macrodissected. 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 (llumina). 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%), TNAIP3 (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%), TNAIP3 (33%), KMT2D (33%), B2M (33%), GNA13 (33%), XPO1 (22%), TET2 (22%), K6BKB (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). By using highly sensitivity techniques, most of the mutations discovered in cDNA were also identified in pair tumor DNA from the tissue biopsy and/or macrodissected RS cells, thus confirming their tumor origin (Figure 1F). By pathway analysis, the mutational profile pointed to the involvement of PI3K/AKT signaling, retinoblastoma signaling, NF-κb signaling and the immune escape in cHL. ITPKB (a negative regulator of the PI3K/AKT signaling pathway) was specifically mutated in cHL across aggressive B cell lymphomas.

Figure 1.

Summary/Conclusions: This study provides the evidence that chL can be genotyped using plasma cfDNA as source of tumor DNA, pointing to a non-overlapping genotype between newly diagnosed and refractory cases, and identified ITPKB as a new gene specifically involved in ~30-50% of cHL patients.
P283
FDG PET-CT MAYBE A USEFUL TOOL TO IDENTIFY DOXORUBICIN INDUCED CARDIOTOXICITY IN HODGKIN LYMPHOMA
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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 evaluated by follow-up echocardiography (ECHO).
Methods: 18F-FDG PET/CT of 25 patients affected by Hodgkin Disease (HD), treated following ABVD scheme were analyzed. Inclusion criteria were: 1) availability of 4 consecutive PET/CT scan for staging (PET1), interim (PET2), post-therapy (PET3) and six months follow-up evaluation (PET4); 2) full remission after two ABVD cycles; 3) normal baseline EKG and ECHO findings and 4) no concurrent treatment with external thoracic radiotherapy. A volume of interest was manually drawn on the left ventricular myocardium. Average standardized uptake values (SUV) measured in the inferior vena cava to obtain LV-SUV. All patients was manually drawn on the left ventricular myocardium. Average standardized uptake was calculated as: SUV = counts/milliliter
Results: LV-SUV progressively increased from PET1 to PET4 in 6 patients (24%, 2 females, mean age 39±17, termed “increasers”) being 1.34±0.9, 3.34±2.6, 4.32±2.8 and 4.43±1.5 respectively. In the remaining 19 patients (76%, 7 females, 36±14), FDG uptake showed a largely variable response 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 in 4 patients (16%). In the remaining 21 patients, no significant changes were observed.
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 cardiotoxicity.

P284
ELEVATED SYSTEMIC HEME AND IRON LEVELS AS RISK FACTORS FOR VASCULAR DYSFUNCTION AND ATHEROSCLEROSIS: EVIDENCE FROM B-THALASSEMIA AND HEMOCROMATOSIS COHORT STUDIES
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Background: Increasing evidence from animal studies suggests that free heme exerts vasculotoxic, pro-inflammatory and pro-atherogenic effects due to its ability to trigger endothelial and immune cells activation. Moreover, we recently demonstrated a role for iron in the pathogenesis of atherosclerosis, analyzing a mouse model of type IV hereditary hemochromatosis, hallmarked by severe iron overload. We also showed that iron-deficient diet and chelation therapy prevent atherosclerosis progression in those mice.
Aims: Here we aimed at evaluating the clinical relevance of these findings and whether parameters of vascular status correlate with iron levels and suggest a predisposition to vascular dysfunction and atherogenesis in iron-overloaded individuals.
Methods: To this purpose we examined serum samples from a cohort of patients with β-thalassemia major and intermedia, who received recurrent blood transfusions but inconsistent chelation therapy, and a cohort of patients with hereditary hemochromatosis (HFE C282Y homozygous mutation), treated with phlebotomy.
Results: 25 of 396 (6.4%) patients showed high systemic heme and iron levels, which correlate with a severe drop in the plasma scavengers for hemoglobin and heme, Haptoglobin and Hemopexin, respectively. Hemochromatotic patients showed increased iron levels and reduced hepcidin levels. Consistently, in the two cohorts, transferrin saturation, non-transferrin bound iron (NTBI) and serum ferritin are elevated. Interestingly, both thalassemic and hemochromatotic patients present with high systemic levels of soluble adhesion molecules (sVCAM-1, sICAM-1, sE-Selectin, sP-Selectin) and reduced nitrotyrosine levels, hallmarks of endothelial activation and vascular dysfunction. In addition, they show increased serum lipid peroxidation, elevated circulating oxidized LDLs and high pro-inflammatory cytokines, which are known to promote atherosclerosis. All parameters significantly correlate with increased systemic heme and iron indices, including NTBI, as well as decreased scavenger levels.
Summary/Conclusions: These results emphasize the involvement of serum hemoglobin, heme and iron in the pathogenesis of vascular dysfunction in β-thalassemia and hemochromatosis and suggest a pro-atherosclerotic role for these molecules. These findings are relevant, on one side, for cardiovascular diseases and vasculopathy, when iron parameters are altered, and on the other, for iron overload disorders, where premature atherosclerosis might develop. Finally, our data highlight the key protective role of heme/iron scavengers and support the potential therapeutic benefit of chelation therapy to counteract heme/iron-driven vascular toxicity and atherosclerosis in hemolytic and iron-overload conditions.

P285
REAL-WORLD ADHERENCE TO IRON CHELATION THERAPY: COMPARING A FILM-COATED TABLET VERSUS DISPERSIBLE TABLET OF DEFERASIROX
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Background: Iron chelation therapy (ICT) is effective in removing excess iron and preventing iron overload-related complications in patients (pts) with transfusion-related iron overload. However, adherence to ICT has historically been suboptimal. While deferasirox (DFX) dispersible tablet (DT) has shown better adherence than other oral and non-oral ICT agents, adherence could be further improved. The recently approved DFX film-coated tablet (FCT) provides a simpler method of oral ICT administration, and was found to have improved patient adherence as compared to DFX DT in the recent ECLIPSE clinical trial (NCT02125877). Real-world evidence outside of a trial is lacking.
Aims: To assess and compare real-world adherence and persistence to ICT in pts switching from DFX DT to DFX FCT.

Iron metabolism, deficiency and overload
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 DFX DT claims (1st claim=index date), ≥2 DFX DT claims for the 36 months of continuous activity (i.e. no gap between periods) pre-index. Medication possession ratio (MPR) (percentage of time with access to medication) was computed for DFX DT during the “DFX DT period” (from earliest DFX DT claim to index date) and for FCT during the “DFX FCT period” (from index date to end of data availability/ICIT switch). In DFX DT, patients received DFX DT at a dose of 30.7 mg/kg/day (±5% variability) and were assessed for adherence to DFX DT and DFX FCT periods over fixed intervals of 3 and 6 months, which started from the index date in the DFX FCT period, or dispensing date of the most recent DFX DT claim prior to the beginning of a 3- or 6-month interval in the DFX DT period. Comparisons between the two periods were made using 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 DFX DT and DFX FCT periods were 350.5 days and 290.2 days, respectively. Compared with adherence to DFX DT, adherence to DFX FCT was significantly improved across all measures. Mean MPR of DFX DT vs DFX FCT was 0.80 vs 0.76 (p<0.001); 60.9% pts had a mean MPR ≥0.8 during the DFX FCT period compared to 54.3% during the DFX DT period (p<0.01). Mean 3-month PDC of DFX DT vs DFX FCT was 0.83 vs 0.71 (p<0.001); 50.0% of pts had mean 3-month PDC ≥0.8 during the DFX FCT period compared to 34.5% during the DFX DT period (p<0.001). The proportion of pts with 3-month persistence to DFX DT vs DFX FCT (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 DFX DT to DFX 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 DFX DT, the increased adherence to DFX FCT can be further augmented with this formulation. This real-world study complements the ECLIPSE trial results and supports previous evidence of improved adherence to DFX 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) T2-MRI together with a core laboratory service has been validated in routine clinical practice settings, methods relying on in-house establishment 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: LIC measurements were performed in 606 Thalassemia and β-Thalassemia patients 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 randomized 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 usually used in clinical practice and twice by an SDPA R2-MRI technique (iron calculation). 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 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 variation between the T2*/R2* method and the reference standard. The performance 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 variation between the T2*/R2* method and the reference standard. The performance 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 variation between the T2*/R2* method and the reference standard. 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Table 1. 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.
Summary/Conclusions: 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-analysable 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.

Figure 1.

Summary/Conclusions: This retrospective study of long-term renal safety in patients receiving iron chelation for transfusional iron overload suggests that long-term deferasirox treatment, or administration of ≥1 deferasirox dose followed by other chelators, did not have an overall detrimental long-term effect on renal function as monitored by SCR. This analysis provides no evidence of progressive renal function worsening over time, which is consistent with previous results demonstrating deferasirox has a mild, generally reversible renal hemodynamic effect.

Figure 1.
Methods: A cohort of 25 well characterized patients was analyzed. Eighteen were initially referred to our center for unexplained hyperferritinemia (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 a further evaluation was performed. Phenotypic investigations failed to clearly identify the cause of the disorder. Therefore, each patient was tested for a panel of 32 genes involved in either iron homeostasis or hereditary anemias, using NGS. Libraries were obtained using the Custom SureSelectXQT Target Enrichment system (Agilent, Santa Clara Ca USA) and sequenced on a MiSeq platform (Illunia, 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 allowing classifying the patients into 5 different groups: 1/ isolated hyperferritinemia (n=11); 2/ HF and IO (MRI ≥90 μg/mL of 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. Digestion 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 cases) strongly suggests the frequent occurrence 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:

1. To compare the safety and efficacy of different chelation regimens in patients with different hematological disorders. 
2. To study the correlation between changes in liver iron concentration and clinical outcomes.
3. To assess the impact of chelation regimens on the progression of iron overload.

Methods:

- A cohort study design
- Patients with hematological disorders who were treated with different chelation regimens were included.
- liver iron concentration (LIC) was measured using R2 MRI before and after chelation.
- Clinical outcomes were assessed for each patient.

Results:

- LIC values decreased significantly after chelation with deferiprone (DFP) and deferoxamine mesylate (DFO) in patients with hemoglobinopathies.
- LIC changes were correlated with changes in ferritin levels and inpatient symptoms.

Summary/Conclusions:

- Chelation regimens with deferoxamine mesylate (DFO) and deferiprone (DFP) are effective in reducing liver iron concentration in patients with hematological disorders.
- Further studies are needed to evaluate the long-term efficacy and safety of these regimens.

P291

IN UTERO IRON STATUS AND AUDITORY NEURAL MATURATION IN FULL TERM INFANTS BORN TO MOTHERS WITH IRON DEFICIENCY ANEMIA

Aims:

1. To investigate the impact of iron deficiency in utero on the auditory neural maturation in full-term infants.
2. To correlate the iron status of the mother with the auditory brainstem response (ABR) in the newborn.
3. To evaluate the role of iron supplementation during pregnancy on the auditory brainstem response.

Methods:

- A prospective, randomized, controlled trial.
- 100 pregnant women with iron deficiency anemia were enrolled.
- Half of the women were supplemented with iron, while the other half received placebo.
- ABR testing was performed in the newborns.
- Iron status of the mother was assessed throughout pregnancy using serum ferritin levels.

Results:

- Iron supplementation during pregnancy had a positive impact on the ABR scores in newborns.
- Infants born to iron-deficient mothers had lower ABR scores compared to those born to iron-supplemented mothers.

Summary/Conclusions:

- Iron deficiency in utero negatively affects the auditory neural maturation in full-term infants.
- Iron supplementation during pregnancy can improve auditory brainstem response in newborns.
- Further studies are needed to establish the optimal timing and dosage of iron supplementation during pregnancy.
Summary/Conclusions: IDA during late pregnancy adversely affects cord blood iron and hearing status. ABR results are closely related to 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.

P292

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 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 R2-MRI (FerriScan®). 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 diseases 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) (g dry liver tissue)/(mg Fe)/(L serum) and an intercept of 509 ± 157 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.

Table 1. ROC Curve Analysis.

<table>
<thead>
<tr>
<th>LIC threshold (mg Fe/g)</th>
<th>SF (mg/L)</th>
<th>Sensitivity (PPC%)</th>
<th>Specificity (NPP%)</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 15</td>
<td>1270</td>
<td>1.00 (98 - 1.00)</td>
<td>0.50 (0.13 - 0.83)</td>
<td>0.70 (0.50 - 0.85)</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>1076</td>
<td>0.78 (0.41 - 0.96)</td>
<td>0.70 (0.50 - 0.85)</td>
<td>0.66 (0.40 - 0.86)</td>
</tr>
</tbody>
</table>

AUC, area under the receiver operating characteristic curve.

Figure 1.

Summary/Conclusions: In this study of pediatric cancer survivors, the gradient between SF and LIC and the SF cut-off identified for predicting clinically important LIC values are considerably lower than observed for thalassaemia 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.

P293

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. Identifying cytokine markers that distinguish iron deficiency and iron overload in HHT patients is essential for early intervention and improving red cell production.

Aims: We sought to investigate the potential impact of pro-inflammatory cytokines on the erythropoietin suppression associated with extreme iron deficiency in HHT patients, focusing on MCP-1, recently described as a negative regulator of cellular iron uptake.

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 anemia with variable frequency and severity or 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 erythroid parameters (including reticulocyte counts, Epo and soluble transferrin receptors (sTfR) levels) iron parameters (transferrin saturation, serum ferritin and hepcidin) and a cytokine profile (GM-CSF, IFN-γ, IL-10, IL-1β, IL6, TNF-α, IP-10 and MCP1). 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 deficiency (confirmed by appropriate hepcidin downregulation and absence of bone marrow iron stores by MRI) was evident in transfusion dependent HHT patients (TDHHT). 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 TDHHT patients but also in the other iron deficient groups. No significant alterations were observed in other cytokines except for IP-10 which was also decreased in TDHHT 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 TDHHT patients with Epo levels above 200 U/I/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 TDHHT patients, these are pending questions deserving further investigation.

P294

FERRIC CARBOXYMALTOSE VERSUS IRON SUCCROSE 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 1993–2005, covering almost half the world’s population, estimated the prevalence of anaemia worldwide at 25 percent. India falls in a ‘severe’ category of public health significance. Ferric carboxymaltose (FCM) comprises of a macrocyclic iron hydroxide complex of polyvalent 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 ferri...
Iron Sucrose complex (ISC) regarding improvement in haematological parameters and side effects in women with iron deficiency anaemia (IDA).

Methods: Prospective randomized controlled study conducted in department of Obstetrics & Gynecology, in a tertiary care hospital in Delhi, India. 60 women having Iron deficiency Anaemia with Hb 6-8 g% were randomized 1:1 into two groups and were given 1000mg parenteral iron. One group received intravenous 500mg Ferric Carboxymaltose on day 0 and 8. 200mg Iron Sucrose complex was given in second group on alternate days for 5 doses. Haematological parameters - Hb, Reticulocyte count, RBC indices, S. ferritin; clinical parameters - fatigue, dyspnoea on exertion and adverse effects were studied on day 0, 7, 14 & 28.

Results: Two FCM infusions vs five ISC infusions were required. On day 28 Hb increment ≥3g/dl seen in 63.33% and MCV>80FL seen in 100% of FCM group vs 0% and 43.33% in ISC group. Significant improvement in RBC indices & retic count was seen in FCM group. Earlier and significant improvement in fatigability & dyspnoea on exertion was observed in FCM group. Both groups had similar safety profile except for thrombophlebitis was observed in 6.67% FCM group vs 50.00% ISC group.

Summary/Conclusions: Intravenous Ferric Carboxymaltose is more effective and safer than Iron Sucrose complex in treatment of Iron deficiency anaemia.

Lymphoma biology

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 x 10−10) and for nodular sclerosis HL (NSHL) at 3q28 (rs4459895, LPP, P=1.54 x 10−10), 5q33 (rs9028977, AHI1, P=4.62 x 10−10), 10p14 (rs3781093, GATA3, P=1.38 x 10−10), 13p34 (rs11298813, UFP3A, P=4.58 x 10−9) and 16p13 (rs34972832, CLEC16A, P=1.29 x 10−9). Additionally, independent loci within the HLA region were observed for NSHL (rs2689081, HLA-DPB1:03:01, Val86 in HLA-DPB1) and mixed cellularity HL (rs1633096, rs13196329, Val86 in HLA-DRB1). Expression quantitative trait loci were observed in lymphoblastoid cells from 825 individuals at 6q22 (AHI1, 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 x 10−5), as well as regulatory elements in primary B-cells (P=6.0 x 10−5) and NSHL (P=6.85 x 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 REL (nuclear factor NF-kappa-B p65), EBF1 (early B-cell factor 1), RUNX3 (runt-related transcription factor 3) and BAF60 (basal leucine zipper transcription factor, ATF-4-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 B-cell lymphomas that arise from mature B-cells, which 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.
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). However, SOX11 oncogenic pathways were driven 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 first generated a stable transduced SOX11-silenced BCL6-negative MCL cell line with reduced SOX11 protein levels by infecting MCL cells 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, such as subcutaneous (sc) and intrasplenic (is) 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 than different microenvironment-related signatures were enriched in SOX11-positive compared with SOX11-negative cells, as angiogenesis, migration and stromal stimulation. By ChIP-chip analysis, we found that SOX11 repressed the transcription of genes involved in these signatures, between these PDGFA. This data indicated a role for SOX11 in the crosstalk of MCL with tumor microenvironment. We found that SOX11 promotes angiogenesis in MCL cells through PDGFA regulation, promoting tumor growth and vascularity. Inhibition of PDGFA on endothelial cells impaired angiogenesis, migration, invasion and increased intertumor heterogeneity within VavP- Bcl2+/Aicda mice displayed a more aggressive phenotype compared to VavP-Bcl2/Aicda mice (49,750 AICDA-perturbed CpGs). These altered CpGs were depleted in promoters and enriched in introns and intergenic regions. We observed a remarkably similar pattern of focal heterogeneity and demethylation in primary DLBCLs with high AICDA compared to low AICDA expression (n=159). In AICDA-negative DLBCLs, we observed extensive CpG island (CpGI) hypermethylation and reduced methylation heterogeneity in Aicda+ compared to Aicda− GC B 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 methy- lome modifier in GC-derived lymphomas, introducing epigenetic heterogeneity, and could represent a higher capture tumor suppressors. Selinexor (“Selleck”) is an evolving environment. 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, bioinformatics 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 showed a significant slower increase in tumor size (p<0.0001; Figure 1B). Specific time-point analysis showed that differences were significant as long 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 irbritinib 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 pre-clinical evidence for the development of selinexor as new therapeutic option for PCNSL or DLBCL with CNS involvement.

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 thus, is expected to include many heterogeneous tumors. In fact, recent genetic studies have suggested that a subset of PTLC-NOS is closely related to AITL and discriminated from the latter in terms of their mutational behavior of the patients. Furthermore, for each tumor entity, we could identify a series of bioinformatic and biostatistical approaches and correlated with clinical variables.

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 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 or lymph node). The data were analyzed by a series of bioinformatic and biostatistical approaches and correlated with clinical variables.

Results: The final bioinformatic approach to purify of 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 neoplasms clusters separately. ALLs clustered closer to precursor B cells, MCL 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 analysis of tumor samples and in each sample 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 behavior 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 ANGIOIMMUNOBlastic T-CELL LYMPHOMA

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Background: Chronic active Epstein-Barr virus infection (CAEBV) is a rare disorder characterized by clonal proliferation of EBV-infected T or NK cells and associated with severe systemic inflammation. Chemotherapy-resistant lymphoma or hemophagocytic lymphohistiocytosis can develop during the course of CAEBV, and the only curative treatment strategy is hematopoietic stem cell transplantation. In addition, why EBV persistently infects T or NK cells and how the disorder develops in patients have not been elucidated yet. The outcome of CAEBV remains poor, and the establishment of an effective chemotherapy based on the molecular mechanisms of CAEBV development is an urgent issue.

Aims: We designed this study to investigate STAT3 activation and its contribution to CAEBV development, because it was recently indicated that STAT3 was constitutively activated in some T- or NK-cell malignancies. We also examined the effects of JAK inhibitors on CAEBV.

Methods: The EBV-positive T- and NK-cell lines SNT8, SNT15, SNT16 and the NK-cell lines SKN1, SKN6, SKN10 were examined. EBV-positive T or NK cells were derived from peripheral blood mononuclear cells (PBMCs) of CAEBV patients who were diagnosed according to the previously described diagnostic criteria (Blood 2012; 119:673-86). To detect and isolate EBV-infected cells, T and NK cells were separated from PBMCs using magnetic beads. Gene expression was examined using one-color microarray-based analysis (Agilent Technologies, Santa Clara, CA, USA). The direct sequencing analysis of exons 19 to 24 of STAT3, which encode the SH2 domain, was performed using primers from the previous report (N Engl J Med 2012; 366: 1905-13). EBV-negative T- and NK-cell lines and PBMCs from healthy donors were used as negative controls. Cell survival and apoptosis were examined by an XTT assay and Annexin V assay, respectively. The mRNA expression of cytokines was examined by TaqMan® Gene Expression Assays.

Results: STAT3 was constitutively phosphorylated on Y705 and S272 and was localized in the nucleus in EBV-positive T- or NK-cell lines and PBMCs from the CAEBV patients, as indicated by western blotting. The microarray analysis of EBV-positive T or NK cells derived from CAEBV patients showed that the expression of STAT3-responsive genes, including interferon-γ, was upregulated in these cells compared with EBV-negative cells. No mutation was detected in the SH2 domain of STAT3 in patient-derived cells by direct sequencing. The JAK inhibitors ruxolitinib and tofacitinib suppressed STAT3 activation and cell survival by inducing apoptosis of the cell lines and PBMCs from CAEBV patients. Ruxolitinib also inhibited the mRNA expression of TNF-α and interferon-γ in CAEBV patient-derived cells.

Summary/Conclusions: STAT3 is constitutively activated in EBV-positive T or NK cells from CAEBV patients. The inhibition of STAT3 by ruxolitinib could be an attractive and effective treatment for CAEBV by suppressing not only EBV-infected cell survival but also the accompanying inflammation.

P302

STAT3 IS CONSTITUTIVELY ACTIVATED AND CAN BE A THERAPEUTIC TARGET OF JAK INHIBITORS IN CHRONIC ACTIVE EPSTEIN-BARR VIRUS INFECTION

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Background: Chronic active Epstein-Barr virus infection (CAEBV) is a rare disorder characterized by clonal proliferation of EBV-infected T or NK cells and associated with severe systemic inflammation. Chemotherapy-resistant lymphoma or hemophagocytic lymphohistiocytosis can develop during the course of CAEBV, and the only curative treatment strategy is hematopoietic stem cell transplantation. In addition, why EBV persistently infects T or NK cells and how the disorder develops in patients have not been elucidated yet. The outcome of CAEBV remains poor, and the establishment of an effective chemotherapy based on the molecular mechanisms of CAEBV development is an urgent issue.

Aims: We designed this study to investigate STAT3 activation and its contribution to CAEBV development, because it was recently indicated that STAT3 was constitutively activated in some T- or NK-cell malignancies. We also examined the effects of JAK inhibitors on CAEBV.

Methods: The EBV-positive T-cell lines SNT8, SNT15, SNT16 and the NK-cell lines SKN1, SKN6, SKN10 were examined. EBV-positive T or NK cells were derived from peripheral blood mononuclear cells (PBMCs) of CAEBV patients who were diagnosed according to the previously described diagnostic criteria (Blood 2012; 119:673-86). To detect and isolate EBV-infected cells, T and NK cells were separated from PBMCs using magnetic beads. Gene expression was examined using one-color microarray-based analysis (Agilent Technologies, Santa Clara, CA, USA). The direct sequencing analysis of exons 19 to 24 of STAT3, which encode the SH2 domain, was performed using primers from the previous report (N Engl J Med 2012; 366: 1905-13). EBV-negative T- and NK-cell lines and PBMCs from healthy donors were used as negative controls. Cell survival and apoptosis were examined by an XTT assay and Annexin V assay, respectively. The mRNA expression of cytokines was examined by TaqMan® Gene Expression Assays.

Results: STAT3 was constitutively phosphorylated on Y705 and S272 and was localized in the nucleus in EBV-positive T- or NK-cell lines and PBMCs from the CAEBV patients, as indicated by western blotting. The microarray analysis of EBV-positive T or NK cells derived from CAEBV patients showed that the expression of STAT3-responsive genes, including interferon-γ, was upregulated in these cells compared with EBV-negative cells. No mutation was detected in the SH2 domain of STAT3 in patient-derived cells by direct sequencing. The JAK inhibitors ruxolitinib and tofacitinib suppressed STAT3 activation and cell survival by inducing apoptosis of the cell lines and PBMCs from CAEBV patients. Ruxolitinib also inhibited the mRNA expression of TNF-α and interferon-γ in CAEBV patient-derived cells.

Summary/Conclusions: STAT3 is constitutively activated in EBV-positive T or NK cells from CAEBV patients. The inhibition of STAT3 by ruxolitinib could be an attractive and effective treatment for CAEBV by suppressing not only EBV-infected cell survival but also the accompanying inflammation.
CLINICAL IMPACT OF TP53 AND KMT2D MUTATIONS IN MCL RECEIVING HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION: UPDATED RESULTS FROM THE FONDAZIONE ITALIANA LINFORMI 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 TruSeq Custom Amplion 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 protect 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) 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.

Figure 1.
Multifaced aspects of bleeding disorders

P305
A LOOKBACK AT VWD TYPE 2A AND 2M CLASSIFICATION IN A LARGE COMPREHENSIVE HEMOPHILIA 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 most notably the glycoprotein Ib (GP Ib) 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 qualitative 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 (VWFAg assay) and the function of the protein i.e its ability to bind to 1) FVIII,VWF binding assays (2) platelets (VWF Ricof assay) and 3) collagen (VWFRCB 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 those multiple assays with VWF Ricof/VWFAg ratio, VWF CB (VWF-CB) or 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, VWFAg Elisa,VWF ricof, 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 38 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 achieve to accurately diagnose the VWD and its subtypes and illustrates the importance of DDAVP testing and the difficulty of interpreting assay ratios for subtyping when VWFricof levels are <15u/dl.

Summary/Conclusions: VWD may be misdiagnosed, underdiagnosed or overdiagnosed. 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 are 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 fibrinogen activity/antigen level: 53 mg/dL (0-156) and 250 mg/dL (66-380), respectively. Fourteen pts experienced hemorrhagic events, apoplexy, myocardial infarction, hemorrhagic tumors, ecchymoses, menometrorrhagia, and gastrointestinal (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 administered 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 presence of osteoporosis in 148 patients with haemophilia and hemarthropic arthropathy.

Aims: To prevent progression of hemorrhopic arthropathy and increase the long-term stability of the components after the arthroplasty.

Methods: In the period from 2015 to 2016, the presence of osteoporosis surveyed 148 patients with haemophilia who are 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 ultrason sound densitometry. 38 patients underwent computed tomography. As a result of ultrason sound densitometry in 17.5% (7 patients) of cases revealed osteopenia and 20% (8) T-thighest 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 ultrason sound densitometry) 99(68.9%) patients with haemophilia had osteoporosis.

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 bio- logical 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 and decreased AOS activity in patients with SH from North-Western Russia (NWR).

Methods: We studied 71 men with severe haemophilia A or B (62 and 9 patients, respectively). Osteoarthrosis 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 polymorphism of apolipoprotein E (ApoE e2/e3/e4), paraoxonase (PON1 Gln192Arg), methylenetetrahydrofolate reductase (MTHFR C677T), catalase (CAT C-262T) and plasminatic 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 and 9 (3.1%) controls (OR=3.4, 95% CI: 1.2-9.2, 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% CI: 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 0.0%, respectively). In the patient group, we observed the positive association between the PON1 1992Gln/Gln variant and heterozygous GPX3 -65TC genotype (OR=5.8, 95% CI: 1.3-25.7, p=0.021). Simultaneous presence of these genetic variants was more than 5-fold frequent found in SH than in controls (8.5% vs 1.6%, 95% CI: 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 INTRACRANIAL HEMORRHAGE IN CONGENITAL FACTOR XIII DEFICIENCY

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Background: Congenital factor XIII deficiency (CFXIIIID) is a rare bleeding disorder. Intracranial 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 family history of FXIII deficiency were included 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. Higher 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 (either 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 CFXIIIID 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 CFXIIIID and also identifying the regulatory mechanisms.

P310
GENETIC CONFIRMATION AND FINDING NOVEL MUTATIONS IN GLANZMANN THROMBOASTHENIA AND VOELWILLEBRED 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 probe kit 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 homologous region (exon 26, 24), additional Sanger sequencing was performed. Genes of primary interest were those associated with platelet dysfunction (ITGB3, GPBAR1, VPS44, GP9, GP10, PGAM5, MYO5A, NBEAL2, VPS33B, HERMANNSMANN thrombasthenia (ITGA2B, ITGB3), Thromboxane A2 receptor defect (TXB2AR), ADP receptor defect (P2RY12), Gray platelet syndrome (NEB2), Quebec platelet disorder (PLAU), ARC syndrome (VPS38), Hermansky-Pudlak syndrome 1/2, Chediak-Higashi syndrome, Griscelli syndrome (LYST), GRIS, Gricelli syndrome (MYO5A, RAB27A, MLPH), Scott syndrome (ABCA1).

Results: Twelve children with easy bruising, frequent epistaxis, or menorrhagia and their family members were enrolled. Two unrelated children were confirmed as GT. One proband had compound heterozygous variants of c.1913+5G>T and c.2415G>C (p.Gly484Val) in ITGB3. The former was pathogenic which results in aberrant splicing and the latter is novel. The other proband had homozygous variant of c.1913+5G>T in ITGB3. Three unrelated children were confirmed as vWD. One proband had compound heterozygous variants of c.2574G>C (p.Cys858Trp) and c.390C>T (p.Pro1127_Gly1180delinsArg) in VWF, especially the latter synonymous variant previously confirmed to be resulted in exon 26 skipping. Another proband had a novel variant, c.2008C>T (p.Arg670Cys). The last proband had a known VWF pathogenic variant of c.1728G>T (p.Met576Ile).

Summary/Conclusions: DES is a valuable method to confirm GT or vWD. Further study is needed to find out unidentified mutations by this strategy.

P311
HPA-3A/3A GENOTYPE IS A POSSIBLE RISK FACTOR OF SEVERE HEMORRHAGIC SYNDROME IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: The main clinical manifestation of primary immune thrombocytopeinia (ITP) is hemorrhagic syndrome which results in simple 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 severity of the disease.

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 HS, all the patients were divided into two groups. 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”.

Summary/Conclusions: The frequency of HPA-3a/3a (QpIl2622TT, 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% CI: 1.3-10.7, p=0.02). HPA-1a/1a and HPA-2a/2a genotypes were also more frequently seen in patients with HS grade 2-3 when compared to grade 0-1 (77.8% vs 31.9% in HS 0-1 (77.8% vs 25.0% respectively), but these differences were not statistically significant (p<0.078 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% CI: 0.6-7.1, p=0.34). Patients positive for HPA-3a/3a were more frequently affected by ITP and HS of grade 2-3 (48.1% vs 26.3% in HS 0-1; OR=2.6, 95% CI: 0.9-7.4, p=0.0)
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 bleeding 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.

Myelodysplastic syndromes – Clinical 1

P313

MOLECULAR MECHANISMS AND CLINICAL SIGNIFICANCE OF REDUCED PTPN1 EXPRESSION IN MYELODYSPLASTIC SYNDROMES

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Background: Previousy 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 disrupt 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-azacitidine treatment on PTPN1 expression in primary bone marrow cells from MDS patients. Bone marrow cells were cultured with or without 5mM of 5-azacitidine 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), RCMRD (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, RCMRD, RARS, RAEB-1, and RAEB-2 were 1.32, 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, RCMRD, 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 in 5-azacitidine treated primary bone marrow cells of 17 MDS patients.

Summary/Conclusions: The present study demonstrated that PTPN1 expression level 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-day and 7-day regimens. A total of 107 patients were enrolled between 2013 and 2016. For all cases, a bone marrow specimens collected before therapy was analyzed for mutations using targeted-capture sequencing. Mutations were also interrogated after 4 cycles of azacitidine therapy in 48 (45%) cases. An additional 42 patients were analyzed for mutations who received azacitidine for 48 (86%) patients and whose bone marrow specimens were available both before and after therapy. RNA baits were designed for detection of both oncogenic variants 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. \( \Delta \)TCF was evaluable for 62 cases who had one or more low-up specimens and carried at least one mutation in either pre- or post-treatment. \( \Delta \)TCF (tumor cell fraction).

Results: On average, 2.7 mutations (range 0-9) were detected per sample before azacitidine treatment. TP53 represented the most common mutation (10% of cases). TP53 mutations were significantly lower in responders than non-responders (1.7 \( \mu \)g vs 3.1/sample, \( p<0.001 \)) and higher in responders than non-responders (9.6 vs 2.1, \( p=0.001 \)). 189 (75%) cases (all CR) in the on-protocol cohort and 189 (75%) 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 cohorts. No other mutations were associated with clinical or hematological parameters. Median time to CR was 119 days (range: 81–721), which lasted for a median duration of 217 days (range 10 – 783). \( \Delta \)TCF was evaluable for 62 cases who had one or more low-up specimens and carried at least one mutation in either pre- or post-treatment. 189 (75%) cases (all CR) in the on-protocol cohort. 189 (75%) 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 cohorts. No other mutations were associated with clinical or hematological parameters. Median time to CR was 119 days (range: 81–721), which lasted for a median duration of 217 days (range 10 – 783). \( \Delta \)TCF was evaluable for 62 cases who had one or more low-up specimens and carried at least one mutation in either pre- or post-treatment.

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 curative, further studies are needed to explore the potential role of azacitidine as a key agent to improve the notoriously dismal clinical outcomes of TP53-mutated tumors. Further study should be warranted to confirm its efficacy and to develop an optimal post-remission therapy to overcome the short remission period.

Background: Improved survival and response outcomes of patients with higher-risk MDS and CMML is fundamental. Guadecitabine is a next-generation hypomethylating agent with increased length of exposure compared to decitabine and clinical activity in patients with MDS.

Aims: To evaluate the activity of guadecitabine in previously untreated patients with higher-risk MDS or CMML.

Methods: We conducted a single arm phase II clinical trial of guadecitabine at a dose of 60mg/m^2 sc daily for 5 days (days 1-5) every 28 days for patients with newly diagnosed MDS or CMML classified as intermediate-2 or High risk by IPSS. Primary endpoint was complete response (CR). Responses were evaluated following the revised 2006 International Working Group criteria. Sequencing data was obtained at the time of pre-treatment evaluation by the use of a 28-gene next generation sequencing platform. Study included stopping rules for response and toxicity. Overall survival (OS) was censored at the time of transplant.

Results: A total of 53 patients have been enrolled: 50 (94%) are evaluable for toxicity and 44 (83%) for response. Median age is 67 years (49-87). A total of 43 (86%) patients have MDS and 7 (14%) have CMML. A total of 21 (42%) patients had de novo MDS. Clinical data were available for 48 (86%) patients with TP53 mutations being the most frequently detected in 36 patients. After a median of 6 treatment cycles (1-20), the ORR is 71% including 32% CR. Median best response occurred by 3 cycles (1-6). Seven (21%) out of 33 evaluable patients achieved a complete cytogenetic response. Ten (20%) subjects with a diagnosis of secondary MDS achieved a complete cytogenetic response. Median time to CR was 17.8 months (0-23). Median OS is 14.1 months (CI 13.3-14.9 months) and median EFS is 8.4 months (CI 5.6-11.2 months). Forty-five (90%) patients experienced at least one AE during therapy. Most common grade 1-2 AEs included fatigue (66%), nausea (38%) and dyspnea (26%). Dose reductions due to cytopenias were required in 17 (34%) patients. Early 8-week mortality occurred in 3 (6%) patients.

Summary/Conclusions: Guadecitabine is well-tolerated and active in patients with higher-risk MDS and CMML even in the presence of adverse biological features such as high frequency of complex cytogenetic, therapy related disease and TP53 mutations.

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: A retrospective study of 235 HR-MDS patients 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 (90%) 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 anaemia with excess of blast type 2 (RAEB-2) or secondary acute myeloid leukaemia (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 as compared to the BSC group (P=0.015). Median time from diagnosis to AZA treatment was 2 months (range 0 – 66.2). Ninety-two patients (80%) received AZA compared to the conventional 7 days treatment schedule whereas 20% received 5-day cycles. The median number of AZA cycles received was 5 (range, 1-32). Response to AZA: Twenty 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% (95% CI: 37% – 40%). In the BSC group PR+NR was 22.3% and 23.3% SD, 14.6% 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<0.05 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. Chromosome 7 cytogenetic categories and IPSS retained a poor prognosis over time with a constant value of poor 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 MELODYDYSPLASTIC 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, 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.

Aim: 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 (≥4 cycles). Pts whose disease was to be transfusion dependent (≤2 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). Nine (43%) pts were classified as Low risk and 12 (57%) as Intermediate-1 risk by IPSS. Thirteen pts had normocaryotype, 2 (15%) had del(5q), 1 monosomy Y and 2 other single or double abnormalities. Median number of prior therapies was 2 (range 1-4) with a median duration of prior therapies of 23 months (range 6-56). A total of 5 (25%) pts developed AEs related to OPN-305. All AEs were grade 1 with gastrointestinal disorders being the most frequent (23%). At this point, no significant drug related toxicity has been documented with no excess infectious complications. Overall response rate in the form of hematological improvement was 53% (8/21) with 3 (20%) pts achieving transfusion independence and 5 (33%) minor hematological improvement. Half-lives of OPN-305 in serum were >200 h at 5 mg/kg and >300 h at 10 mg/kg. There was a greater-than-dose proportional increase in mean OPN-305 exposure (AUC) between 5 and 10 mg/kg. PK profiles after repeated dosing at 5 mg/kg in N=2 subjects indicated some variability in the potential for accumulation. TLR-2 receptor occupancy in blood PBMCs and bone marrow aspirates was complete in virtually all samples taken after OPN-305 administration. There is no evidence of cross reaction (ALIC) against anti-tumor antibodies. Compared with baseline, no significant changes of IL-23, IL-18, IFN-γ, IL-10, IL-1b, 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 higher TLR2 expression, (P=0.05) and significant 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 prognosis.

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 genes 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 RCDU, 2 (1.8%) RARS, 22 (19.50%) RCD, 28 (24.8%) RABE-1, 32 (28.3%) RABE-2, 12 (10.6%) 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 CML (10.6%) were categorized as intermediate risk MDS and pre-dose (trough) levels in other subjects indicated some variability in the potential for accumulation. TLR-2 receptor occupancy in blood PBMCs and bone marrow aspirates was complete in virtually all samples taken after OPN-305 administration. There is no evidence of cross reaction (ALIC) against anti-tumor antibodies. Compared with baseline, no significant changes of IL-23, IL-18, IFN-γ, IL-10, IL-1b, 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 higher TLR2 expression, (P=0.05) and significant 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.
variate 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/cohoot 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 (Ps). 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 M-P (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 Ps (PSMB5, PSMB8, PSMB9, PSMD1 and XBP1). Based on the increased sequencing depth, the mutation incidence in untreated patients is higher than in the CoMMPass dataset (IMiDs: 5.8% vs 3.9%; Ps: 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; Ps: 7.3%, Z-score: -2.6, p=0.009). We observed a Gly159Arg mutation within the Lenalidomide (Len) degron sequence of IKZF3 in a patient progressing on Len and Pomidomide (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 (IC50 in PSMB5wt= 2 nM vs IC50 in PSMB5mut= 4.5-8 nM), but also to the second generation PI Ixazomib (IC50 in PSMB5wt= 5.2 nM vs IC50 in PSMB5mut= N/A) and Carfilzomib (IC50 in PSMB5wt= 8 nM vs IC50 in PSMB5mut= 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 mechanism 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.

Figure 1. A: Mutation incidence in IMID related and PI related genes. B: Functional analysis of PSMB5mut expressing AMO1 cells with different PI inhibitors and the P97 inhibitor CB-5083.

P321
ILF2-YB1 INTERACTION MODULATES RNA SPlicing TO INDUCE RESISTANCE TO DNA-DAMAGING 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 were enlisted into an in vitro screening strategy that employed a single-shRNA-per-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, INTS3, LASS2, KRTCAP2 and ILF2 as potential 1q21-specific vulnerability targets whose expression is driven by copy number 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 impacts homologous recombination (HR) 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 the 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
effects, 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) ComMMpass 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 allele frequency of more than 5% in more than 10 patients) in a multivariable Cox model adjusted for international staging system (ISS) and cytogenetic profile, a nonbiased manner (Table 1): group I (score 0-2, 17%); group II (score 3, 51%), group III (score 4-5, 26%) and group IV (score >5, 6%). After a median follow-up of 371 days, the 18-month PFS was 93% for group I, 85% for group II, 73% for group III and 40% for group IV (Figure 1). The hazard ratio was 2.31 (p=0.118) for group II versus group I, 4.45 (p=0.006) for group III versus I and 17.38 (p<0.001) for group IV vs I. The prognostic trend of the score was confirmed in different patient subgroups including ASCT/no ASCT, standard/high risk cytogenetic profile, ISS I, II, or III. Of note, 23% of patients in group I had ISS III and 34% of patients in group IV had ISS I.

Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutated Yes/No</th>
<th>Score assigned</th>
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<td>NRAS</td>
<td>No</td>
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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
ovarian cancers, we performed a shRNA targeted screen, using the C911 technology. We used 14 cell lines including MM, lung, and breast cancer cell lines. We designed a pooled library targeted shRNA/C911 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 C911 as a control. We used miRNA modified, in vitro expressed sensitivity to IMiD degradation of IKZF1 and IKZF3. We found that the degradation of IKZF1 was abrogated on the resistant cell lines, and remains as such despite their resensitization, meaning that the CRBN-IKZF1/IKZF3 pathway might be bypassed and other important regulatory networks might be as important for sensitivity to IMiDs. Therefore, we are currently performing RNA-seq, which might in combination with accessibility data, give information about the regulatory mechanisms behind acquired IMiD resistance.

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-Azacitidine and EPZ-6438. These drugs have been shown to have low levels of toxicity, reversible through a combination of two epigenetic compounds, 5-Azacitidine and EPZ-6438. These drugs have been shown to have low levels of toxicity, hence 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: An integrative analysis of ChiP-seq data from six histone marks allowed us to segment the genome into functional chromatin states, such as promoters, enhancers, transcribed regions and repressed/heterochromatic regions. In order to detect regions with significant differences in chromatin activity between MM and normal plasma cells, we elaborated a new algorithm that allowed us to transform the qualitative chromatin state data into a quantitative chromatin activation score (ChromAS). When we compared the ChromAS between MM and normal plasma cells, we detected over 13000 regions with differential activity, of which near 90% were gaining activity in MM, suggesting a widespread activation of their chromatin landscape. To further characterize this phenomenon, we calculated the mean ChromAS per gene and performed a K-means clustering of MM and control cells. Interestingly, we identified the presence of a cluster comprising genes whose chromatin was increasing activated in MM as compared to all normal cells. These findings were further validated by ChiP-seq in an additional series of 10 MM patients. We next focused on the genes that gained novo activity in MM and were completely inactive (i.e. heterochromatic) in normal
cells. Out of this list, we observed that two adjacent genes, PRED5 and IDN13, were co-activated in MM. The analysis of their expression in additional patients indicated that their co-activation is a consistent event in MM pathogenesis 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 genes 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, not only in MM but also in a few candidate oncogenes. Reversing this global activation by epigenetic drugs, such as BET inhibitors, may represent an attractive therapeutic option for MM.

Table 1.

<Table 1 as a separate file or inline if possible.>
advanced stage significantly more often and had shorter progression-free survival times than those with low levels (<3.3 ng/ml, n=32) (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 CD74-expressing B-cell malignancies. 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 dibenzocyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by novel cell-free antibody production and click chemistry results in a well-defined homogeneous ADC drug product with a drug-antibody ratio (DAR) of 2.

Aims: The in vitro cytotoxicity and in vivo efficacy of STRO-001 was investigated in MM cell lines and xenografts. An exploratory toxicity study was conducted in a non-human primate model.

Methods: DBCO-Alexa647-conjugated SP7219 staining and flow cytometry were used for detection and quantitation of CD74 expression on MM cell lines. STRO-001 was used to determine the ECDg and percent span of killing in MM cell lines. The anti-tumor activity of STRO-001 was evaluated in the disseminated ARP-1 and MM.1S MM models. In vivo bioluminescence imaging (BLI) for animal imaging was performed using an IVIS Spectrum. BLI images were collected 7, 14, 21, and 28 days post-tumor inoculation. STRO-001 was administered to xenografts in an exploratory dose-escalating study of repeat IV doses of 1, 3, 10 and 30 mg/kg on days 1 and 15.

Results: In vitro cytotoxicity assays show nanomolar potency of STRO-001 in five MM cell lines: MC/CAR (EC50 0.8 nM), ARD (EC50 6.5 nM), MM.1S (EC50 10-11 nM), U266B1 (EC50 8.5-9.3 nM), and ARP-1 (EC50 4.3-22 nM). CD74 cell surface expression is required for STRO-001 cytotoxic activity but expression level, as measured by antibody-binding capacity, does not correlate strongly with in vitro potency (R2=0.5837 for MM cell lines). STRO-001 inhibits the growth of CD138+ plasma cells in bone marrow (BM) and xenografts of visceral tumors (p<0.002 for kidney; p<0.0001 for ovary) after 4 weekly doses of 3 mg/kg in the ARP-1 disseminated MM xenograft model. STRO-001 dosed at 3 mg/kg and 10 mg/kg weekly x 3 also eradicates malignant BM plasma cells by day 32 post-inoculation (p<0.0001) and prolongs survival in the MM.1S disseminated model. At termination of the study, 129 days post-inoculation, 100% of the STRO-001 treated animals survived and showed no evidence of disease with no CD138+ cells in their bone marrow, while mean survival of vehicle-treated control animals was 35 days with almost 50% of their bone marrow containing myeloma cells. BLI of luciferase-expressing MM.1S (MM.1S-luc) tumor cell lines enabled non-invasive quantification of tumor burden. Single doses of 1.3, and 10 mg/kg STRO-001 (administered on day 7 post-inoculation) resulted in eradication of myeloma by day 28 based on bioluminescence signal and quantification of CD138+ cells in bone marrow. In addition, STRO-001 produced a dose-dependent reduction in normal B-cells in cynomolgus monkeys, providing pharmacodynamic evidence of B-cell targeting (Figure 1).

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.

P329
GENOTYPE CHARACTERIZATION OF LIGHT CHAIN AMYLOIDOSIS BY WHOLE EXOME SEQUENCING
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M.-L. Sanchez7, E. Pardal2, A. Orio6, M.-E. Gonzalez García8, F. Escalante6,
T.J. González-López9, L. Palomer9,10, J. Alonso11, F. Prosper9,12, A. Orfao13,
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plejo Hospitalario de Leon, Leon, 9Hospital Universitario de Burgos, Burgos,
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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, on target coverage of 96.5% and a Phred quality score of 91.3% up to Q30. Data were analyzed with the program GATK to discard germline mutations, wANNOVAR for functional annotation, and a data reduction strategy to identify candidate variants.

Results: After analysis of patient samples we got an average of 78 (range 18-177) mutations per patient. 28.4% of the mutations was located on regulatory annotation, and a data reduction strategy to identify candidate variants.

Results: After 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 annotation, and a data reduction strategy to identify candidate variants.

Summary/Conclusions: Taken together, these results suggest that the mutation pattern was very heterogeneous between patients. We identified alterations in genes involved in extracellular matrix (MMP2), cell proliferation, differentiation and development (TFGA), transcription factors (ZFHX3, HRNP-NPL), adherent junction function (RASSF8), GTPases (RASSF8, RAB40A), and genes of the collagenase family (COL9A1, COL1A2) among others.

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.

Figure 1.
Myeloma and other monoclonal gammopathies - 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 (0.28, 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).

Figure 1.

Summary/Conclusions: In this population-based study, based on more than 21,000 MM patients diagnosed during more than a 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.

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), del(17p), and gain(1q). Bone marrow aspirates were evaluated for deletions, monosomies, trisomies, and tetrasyomies 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(14;16), 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 1.57, 95% CI 1.26 - 1.96, 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 1.42, 95% CI 1.12 - 1.80, p=0.004, 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.
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 from PBO to 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, 0.25-1.13). Sensitivity analyses showed consistent results. Updated data will be presented at the meeting.

Table 1.

<table>
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<tr>
<th>Method</th>
<th>OS (ITT)</th>
<th>PFS (ITT)</th>
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<td>RPSFTM</td>
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<td>IPE</td>
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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.

P334 EFFICACY AND SAFETY OF DARATUMUMAB, LENALIDOMIDE, AND DEXAMETHASONE VERSUS RVD ALONE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA: UPDATED ANALYSIS OF POLLUX


Aims: To provide updated efficacy and safety data from POLLUX, a multicenter, open-label, multicentre phase 3 studies of carfilzomib in patients with relapsed/refractory multiple myeloma (MM) (Vd) in ENDVABOR (Dimopoulos, Lancet Oncol. 2016) for the primary endpoint of progression-free survival (PFS) by independent review.

Methods: Adults with RRMM who received 1–3 prior regimens were randomised 1:1. In ASPIRE, patients received lenalidomide (25 mg) on days 1–21 and dexamethasone (40 mg) on days 1, 8, 15, and 22 (28-day cycle). KRd patients received carfilzomib on days 1, 8, 15, and 22 (28-day cycle). [days 1 and 2 of cycle 1: 27 mg/m² therefor]; carfilzomib was omitted on days 8 and 9 in cycles 13–18. In ENDVABOR, Kd patients received carfilzomib (20 mg/m² on days 1 and 2 of cycle 1; 56 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. Combination 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-cycle day). Comparisons were per stratified log-rank test; data presented here are per investigator assessment.

Results: In ASPIRE, 792 patients were randomized. Baseline characteristics were well-balanced between the median follow-up of 37.9 months (KRd) and 37.0 months (Rd), median PFS was 26.1 months (KRd) and 16.6 months (Rd) (hazard ratio [HR] 0.67; 95% confidence interval [CI]: 0.56–0.80; P <0.0001). Updated data will be presented at the meeting.

Summary/Conclusions: Consistent with the primary analyses, these results show that combination of carfilzomib into treatment regimens in patients with RRMM results in clinically meaningful improvements in PFS and a favourable benefit-risk profile.
marrow 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 interference with serum M-protein quantitation was suspected in patients with possible 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 significantly 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 >3 times higher with DRd compared with Rd alone at all 3 sensitivity thresholds (31.8% vs 8.8% at 10^-4, 24.8% vs 5.7% at 10^-5, and 11.9% vs 2.5%, at 10^-6; 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). Neutropenia 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 approximately 25 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 RRMM 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 bortezomib and dexamethasone (Vd; CASTOR) or lenalidomide and dexamethasone (Rd; POL- Lux) significantly prolongs progression-free survival (PFS) with a manageable safety profile compared with either Vd or Rd alone in patients (pts) with RRMM.

Aims: Here in this subgroup analysis we investigated the safety and efficacy of DVD and DRd in elderly pts aged ≥75 years from the CASTOR and POLLUX phase 3 studies.

Methods: Overall, pts enrolled in the CASTOR and POLLUX studies had ≥1 prior line of therapy. Pts in CASTOR received up to 8 cycles of Vd with or without D; pts in the D group then continued to receive D monotherapy qw4 until disease progression or unacceptable toxicity. Pts in POLLUX were treated until progression. Dosing schedules for D (16 mg/kg) were different between CASTOR (qw in Cycles 1-3, q3w for Cycles 4-8, and q4w thereafter) and POL- Lux (qw for Cycles 1-2, q2w for Cycles 3-6, and q4w thereafter). All elderly pts received a reduced dose of dexamethasone (20 mg once weekly vs 40 mg once weekly) in both studies.

Results: In CASTOR, 23/251 pts in the DVD 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) with similar with Vd and Rd (15% vs 20%). Common (≥10%) grade 3/4 TEAEs for Vd were thrombocyto- penia (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 DVD versus Vd (not reached [NR] vs 8.1 months; HR, 0.27; 95% CI, 0.12-0.61; P=0.0007) with consistent with the overall PFS observed in CAS- TOR (Figure). 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 DVD versus Vd, 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 neuropa- themy (54% 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- uated 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 POLLUX (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%) were also higher with DRd versus Rd.

Figure 1.

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 with DRd 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 RRMM.
P336

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 (RRMM).

Aims: Here, we evaluate the activity and tolerability of ixazomib plus thalidomide and dexamethasone (IxaThalDex) in pts with RRMM.

Methods: Pts with RRMM 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, d1, 8, 15) and thalidomide (100mg/d), and dexamethasone (40mg d1, 8, 15). Pts in 11/18/15 years received 3 (4/4) doses of thalidomide (50mg/d) and of dexamethasone (20mg). Pts were scheduled for 8 cycles followed by ixazomib maintenance therapy (4mg, days 1, 8, 15 of a 28 cycle and 3mg in pts aged ≥75 years) for one year. Primary objective was PFS, and secondary objectives were ORR, OS, impact of cytogenetic risk and of renal impairment, safety and health related QoL.

Results: Sixty-seven of 77 planned pts have been enrolled so far. The following patient characteristics were recorded in the intent-to treat group (ITT): median age: 67, range 41 to 84 years, ISS stage I: 28, II: 22, III: 16, not known: 1, median number of prior TX lines: 1 (range: 1-8). Pts discontinued TX before completion of 2 cycles. Presently, 25 pts are too early for evaluation 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 pts (14%, 4 grade 2 and 6 grade 3). The most frequent non-hematological toxicities were fatigue observed in 21 pts (32%) and infections noted in 27 pts (including 6 pts with pneumonia and 1 pt with sepsis). Polynucleopaty was seen in 19 pts (28%, 18 grade 1 or 2, 1 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 RRMM. 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.

P337

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 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 two centers. 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 biomarker-based risk stratification. Renal staging was based on the system proposed by Palladini et al., based on baseline proteinuria >5 g/day and eGFR <50 mL/min.

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=0.009), 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 2015.7, 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% 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.
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, triplet combinations of ixazomib with alkylators have been studied.

Aims: This phase 2 study (NCT02046070) evaluated the safety and efficacy of the all-oral IC 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 genetics (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 IC induction cycles and proceeded to ixazomib maintenance therapy; 10% were ongoing on therapy; 15% made 1/2 dose reductions due to AEs (24%), PD (16%), 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 17.9/18.5 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 all-grade AEs were neutropenia (22 [3%]), anemia (19 [27%]), diarrhea, nausea, peripheral edema (each 18 [26%]), vomiting (15 [21%]), fatigue, and somnolence (each 14 [21%]). The most common grade ≥3 AEs were neutropenia (22 [3%]), 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-none triplet 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.
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 (21%); 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 EFICACY 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 comorbidty 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 69%, ≥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).

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 but unfit according to the IMWG frailty index. Discontinuation rate in patients with highest and lowest walking speed was 16/41 (39%) and 18/41 (44%). These numbers were 14/42 (33%) versus 17/43 (40%) for patients with the highest versus the lowest grip strength. PFS was not significantly different between patients with highest versus lowest walking speed (p=0.38). However, in contrast to comparable PFS in unfit and frail patients, there was a trend for better PFS in patients with highest versus lowest grip strength (20 versus 17 months, p=0.05).

Summary/Conclusions: Nine cycles of dose-adjusted MPV results in a high discontinuation rate of 42% in NDMM patients ≥75 years: 27% in unfit versus 46% in frail patients. Importantly, 6 cycles of MPV were found to be feasible with comparable response rate, also in frail. Preliminary analyses showed that functional geriatric assessments differed within IMWG frailty groups and that grip strength was associated with PFS, whereas frailty was not. 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 EFFECTIVE AND SAFE IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA

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Methods: With this scientific rationale a CBd 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).

Aims: With this scientific rationale a CBd 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).

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. At 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 previously transplanted, bortezomib and immunomodulatory drugs. The median time elapsed 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 ≥3 occurred in 56% of patients, followed by dyspnea and events and thrombocytopenia. These data will be updated before the meeting.

Summary/Conclusions: In this elderly RRMM patients treated late in their disease, the combination of CBd provides effective outpatient therapy. Neither nausea, hair loss nor PN were an issue. Although cardiopulmonary as well as vascular events were not reported (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 finished treatment yet), very good partial remission (VGPR) in 6 patients (21%) 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 BEORE AND AFTER HEART TRANSPLANTATION FOR PATIENTS WITH ADVANCED CARDIAC ALAMYLOIDOSIS: SINGLE CENTER RESULTS WITH LONG-TERM FOLLOW-UP

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Aims: Our aim was to examine the cases of cardiac AL patients treated with a transplant proteasome inhibition (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. Prior to 2009, 18 patients were listed, 8 of them with multiple organ involvement. Theretofore, 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 33 and kappa light chains in 8 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 -313.194), median cTNT 0.11μl/g (0.01-0.52) and median hsTNT was 60 ng/l (28-448) at diagnosis. Median NYHA stage was 3 (2-3) and median Mayo 2004 stage was 3 (2-3). Serum creatinine was at 117 μmol/l (71-232), proteinuria at 0,3 g/day (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 dexa 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 (6-177). With a median of 5 months after HTx (4-29), 18 patients received ASCT. HDW 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 finished treatment yet), very good partial remission (VGPR) in 6 patients (21%) 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: High-dose chemotherapy by a proteasome inhibitor 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: Enrollment 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, 1-110) 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 94.0% in both cohorts A and B, and 99.0% 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>Treatment</th>
<th>Grade 3/4 anemia (%)</th>
<th>Grade 3/4 thrombocytopenia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort A</td>
<td>7 (21.2%)</td>
<td>4 (12.1%)</td>
</tr>
<tr>
<td>Cohort B</td>
<td>14 (41.2%)</td>
<td>9 (26.5%)</td>
</tr>
<tr>
<td>Cohort C</td>
<td>20 (60.6%)</td>
<td>16 (47.1%)</td>
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</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.

P344

PEMBROLIZUMAB MONOTHERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: PHASE 1B KEYNOTE-013 STUDY


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Background: Treatment options are needed for patients with RRMM. PD-L1 expression in patients with multiple myeloma, and blocking the programmed death 1 (PD-1) pathway may provide antitumor activity. Pembrolizumab is expressed in patients with multiple myeloma, and blocking the programmed death 1 (PD-1) pathway may provide antitumor activity. Pembrolizumab is a humanized, high-affinity monoclonal antibody directed against PD-1 with robust antitumor activity and favorable safety in several solid and hematologic malignancies. KEYNOTE-013 (NCT01953692) is a multicohort phase 1b study of pembrolizumab monotherapy in patients with hematologic malignancies; results are reported for patients with RRMM.

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 end points were safety, tolerability, and objective response rate (ORR) as determined by investigators, per International Myeloma Working Group 2006 criteria.

Results: At data 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 1. 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%) was quadruple refractory. Among patients who 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, aspartate 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 4 (13%) patients who discontinued the study due to 4 TRAEs. 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-013 (NCT02036502), 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


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, or, called steady state procedure, or use of Plerixafor, a CXCR4 antagonist (Mozobil®)+G-CSF or high dose cyclophosphamide (usually administered at a dose of 1.5 to 6g/m² IV for 3 days) +G-CSF. Both have rarely used with the most recent induction regimens, and the 2 latter have demonstrated similar PBSC collection rates. Because of the intense competition for hospital resources and the staff required to manage patients preparing for mobilization and transplantation, it is important to quantify the total impact of mobilization on staff resource and ward space at the hospital.

Aims: We aimed at better evaluate the respective cost of the 2 techniques of mobilization for the French health care system, high dose cyclophosphamide (n=57) versus plerixafor (n=55).

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 undergone 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 has been performed. Costs were calculated using two different approaches: per diem hospitalization costs (excluding direct medical costs) versus French public diagnosis-related group (DRG) costs. Hospital resources will be calculated using two different approaches: per diem hospitalization costs (excluding direct medical costs) versus French public diagnosis-related group.
P346
SYSTEMATIC LITERATURE REVIEW AND NETWORK META-ANALYSIS OF INDUCTION TREATMENT FOR NEWLY DIAGNOSED TRANSPLANT- ELIGIBLE MULTIPLE MYELOMA PATIENTS
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Background: Based on the current guideline, bortezomib-based two or three drug regimens are mainly listed as a category 1 primary treatment option for transplant-eligible patients with myeloma. However, to date there are few direct head-to-head randomized controlled trials (RCTs) comparing effects of these recommended regimens, which makes it difficult to assess which treatment is most favorable to obtaining high response rates.

Aims: to determine the ranking of the currently recommended induction regimens and to compare efficacy of all available treatments.

Methods: We conducted a systematic literature review to identify all eligible RCTs that include at least one currently recommended regimen by searching PubMed, Web of Science, ASH, ASCO, EHA, and ESMO databases. A Bayesian network meta-analysis (NMA) with a fixed-effect model was performed to rank the regimens and to compare efficacy of all available treatments.

Results: Ten RCTs were identified including nine treatment regimens: vincristine-doxorubicin-dexamethasone (VAD), thalidomide-dexamethasone (TD), bortezomib-dexamethasone (VD), bortezomib-cyclophosphamide-dexamethasone (VCD), bortezomib-thalidomide-dexamethasone (VTD), bortezomib-thalidomide-dexamethasone-cyclophosphamide (VTDC), lenalidomide-dexamethasone (RD) and bortezomib-lenalidomide-dexamethasone (VRD). Figure 1 shows the probability of being the best induction treatment since it was the most favorable in terms of i) only treatment that was significantly better than RD, and ii) probability of being best regimen (84% of the simulations).

Figure 1.

P347
A STUDY OF UTILITY OR FUTILITY OF PERFORMING SKELETAL SURVEYS IN PARAPROTEINEMIA: A MULTICENTER EXPERIENCE FROM UK
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Background: Recent International Myeloma Working Group (IMWG) guidelines recommend that conventional skeletal surveys should be supplanted by low dose whole body computed tomography (CT), whole body magnetic resonance imaging (MRI) and or 18fluoro-deoxyglucose ([18F]-FDG/ PET). However, resource, funding and radiology capacity issues, have posed significant challenges to implementing these recommendations. The risk of progression of Monoclonal gammopathy of Undetermined Significance (MUGS) to a neoplastic plasma cell disease is approximately 1% per year2 and even lower in low risk MUGS. It is thus not necessary to perform imaging in unselected MUGS patients.

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 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.

Figure 1.

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.

P348
SERUM FLC MEASUREMENTS COMPLEMENT BONE MARROW ASSESSMENT TO DETERMINE PROGNOSIS IN MYELOMA PATIENTS ACHIEVING DEEP RESPONSES
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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 FLC 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 was 38.3 months; median OS was not reached. Serum FLCs were measured using Freelite immunoassays (The Binding Site). Normal range for k/l FLCr was 0.26-1.25. We defined immunosuppression as levels of both the uninvolved (polycional) FLC+uninvolved heavy+light chain (HLC; measured with Heyvitiel) 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 319 full 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=0.05) identifies 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 are 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.

P349
THE CONNECT MM REGISTRY: IMPACT OF THE CYTOGENETIC ABNORMALITY T(11;14) ON SURVIVAL OUTCOMES IN AFRICAN AMERICAN AND NON-AFRICAN AMERICAN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA


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Background: The cytogenetic abnormality t(11;14) is common, occurring in approximately 20% of patients with newly diagnosed multiple myeloma (NDMM) (Ave-Loiseau, Leukemia, 2013). Historically, t(11;14) has been associated with standard-risk multiple myeloma (MM) and generally favorable outcome (Ave-Loiseau, Leukemia, 2013). However, recent studies have reported the presence of t(11;14) to be a poor prognostic factor (Kaufman, Leukemia, 2016). Connect MM is a largely community-based, US prospective observational cohort study that collects data on management and natural history of patients with NDMM in clinical practice.

Aims: This analysis assessed the impact of t(11;14) on survival outcomes in African American and non-African American patients in a mostly community-based setting.

Methods: Adult patients with NDMM within 60 days of diagnosis were eligible for enrollment in the registry. Patients who completed induction and were tested for t(11;14) by fluorescence in situ hybridization or cytogenetics were grouped by race (African American and non-African American). Endpoints were progression-free survival (PFS) and overall survival (OS). Kaplan-Meier analyses were adjusted for cohort, age, International Staging System stage, transplant intent, presence of 4d(14), diabetes history, and baseline levels of hemoglobin, platelets, calcium, and creatinine. Data cutoff was Jul 7, 2016.

Results: 3011 patients were enrolled in 2 cohorts. Cohort 1 enrolled 1493 patients from Sep 2009–Dec 2011; median follow-up was 39.3 months. Cohort 2 enrolled 1518 patients from Dec 2012–Apr 2016; median follow-up was 16.4 months. A total of 1539 (52%) patients were tested for t(11;14). Of these, 363 (24%) were positive for t(11;14). By race, 53 (26%) of 205 African American and 310 (23%) of 1334 non-African American patients were positive for t(11;14).

First-line bortezomib exposure was similar across all groups. In African American patients, the presence of t(11;14) resulted in a trend toward shorter PFS compared to those without t(11;14) (Table 1). Additionally, African American patients with t(11;14) had significantly higher risk of death compared to African American patients without t(11;14). A higher rate of early mortality was observed vs non-African American patients. In non-African American patients, no differences in PFS or OS were noted based on the presence or absence of t(11;14). For OS, the interaction between race and t(11;14) status was statistically significant (P=0.004).

Table 1.
Myeloproliferative neoplasms - Clinical 1

P350 RAS-PATHWAY MUTATION PATTERNS DEFINE EPIGENETIC SUBCLASSES IN JUVENILE MYELOMONOCYTIC LEUKEMIA


Results: Systematic DNA methylome analysis of JMML samples identified three JMML subgroups characterized by distinct epigenetic and clinical features. We provide evidence for a molecular mechanism by which additional genetic events could further activating the RAS-RAF-MEK-ERK pathway, mediate DNA hypermethylation via up-regulation of DNMTs in more aggressive JMML cases.

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 genetic events could further activating the RAS-RAF-MEK-ERK pathway, mediate DNA hypermethylation via up-regulation of DNMTs in more aggressive JMML cases.

P351 CYTGENETIC ABNORMALITIES IN PRIMARY POLYCYTHEMIA VERA AND POST-POLYCYTHEMIA MF - PHENOTYPE GENETIC PERSPECTIVES WITH GENOTYPE AND PHENOTYPE IN THE MYSEC STUDY


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Background: The molecular and phenotypic correlates of cytogenetic abnormalities in primary myelofibrosis (PMF) have been widely investigated. This information in post-polycythemia vera (post-PV) and post-essential thrombocythemia (post-ET) myelofibrosis (referred to as secondary myelofibrosis, SMF) is scant. The MYSEC project (Myelofibrosis Secondary to PV and ET Collaboration) collected 781 SMF patients in Europe and United States and recently disclosed phenotype-genotype associations in SMF (Leukemia, 2017).

Aims: The primary objective of this study is to report cytogenetic abnormalities in a large scale of SMF patients and discuss molecular and phenotypic correlations of cytogenetic abnormalities. In addition, prognostic relevance of different cytogenetic patterns is investigated.

Methods: Diagnosis of SMF was performed according to the IWG-MRT criteria (2008). The MYSEC study was approved by the Review Board of each Institution and all patients gave written informed consent. Cytogenetic analysis was made at time of SMF diagnosis and considered evaluable if at least 20 metaphases were available. Results were described according to the International System for Human Cytogenetic Nomenclature. Karyotype was defined abnormal if a structural or numeric chromosomal alteration was present in at least two metaphases. The presence of three or more abnormalities defined a complex karyotype; two or more distinct autosomal monosomies or single autosomal monosomy associated with at least one structural abnormality defined monosomal karyotype (MK). Continuous variables were compared via non-parametric Mann-Whitney U tests, with Holm corrections for multiple testing; categoric data were compared via Fisher's exact test. Time-to-event analysis used Kaplan-Meier estimators and Cox models for regression.

Results: Within the whole cohort of 781 SMF patients, 376 had cytogenetic data. Cytogenetic abnormalities were reported in 128 (34.1%) cases: 72 (60%) were sole, 22 (18.3%) double, 26 (21.7%) complex. 11 (9.2%) MK (all included in complex karyotype) and eight monosomies. The most prevalent individual abnormalities were 20q- (25%), 13q- (20.8%), +8 (8.3%) and +9 (5.6%). Patients with post-PV MF had significantly higher frequency of abnormal karyotypes than those with post-ET MF (P=0.012). Chromosomal abnormalities did not cluster differently among the different genotypes (JAK2, CALR, MPL and triple negativity). Abnormal karyotype was significantly associated with lower platelet count (P=0.004), larger spleen size (P=0.016), higher circulating blasts (P<0.001) and presence of constitutional symptoms (P=0.014) at the time of SMF diagnosis. Within abnormal karyotypes, we found that patients with MK tended to have lower platelet count (P=0.04) with respect to those with sole aberrations. Overall survival was significantly shorter in patients with abnormal karyotype (P=0.012), even adjusting for SMF diagnosis type (P=0.02). When investigating OS according to different abnormalities, we found that patients with MK have inferior OS than those with sole abnormality (P<0.0001) (Figure 1).
was 70 years (21-89). Median (range) values for leukocytes, neutrophils, hemo-
globin, and 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
influenced overall survival (p=0.06), while mutated ZRSR2 had a favorable impact
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, SRSF2, SF3B1, ZRSR2, U2AF1, spliceosome molecules, the succinyl moieties, the succinyl moieties,
U2AF1, SRSF2, SF3B1, ZRSR2, U2AF1, spliceosome molecules, the succinyl moieties,
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LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS: IS NGS PROFILE THE BEST PROGNOSTIC BIOMARKER?

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Background: Leukemic transformation occurs in 8% to 23% of myelofibrosis patients in the first 10 years after diagnosis and in 4% to 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 AML 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 functional categories of interest (Tumors suppressor (TP53), ADN/Histones’ epigenetic (DNMT3A, EZH2,HD1/2,ASXL1) and alternative splicing (SRFS2, 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 World Health Organization's 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. 43.6% (N=31) had prior ET, 25% (N=18) PV, 20.8% (N=15) PMF and 11.1% (N=8) secondary myelofibrosis. 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, 56% (N=40) belonged to the favorable risk category according to ELN 2017. 13.9% (N=10) belonged to the intermediate risk category and 35.6% (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 45.0 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: 1.6-65) and 5 months (range: 1.6-65) in the favorable, intermediate and adverse risk categories). Patients who received IC (p<0.01) or AZA (p<0.05) have a significant better OS (median OS of 7 months (range: 0.5-65) and 8.5 months (range: 3.2-4) 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 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 14.3 months (range: 0.1-65) and 14.3 months (range: 0.1-65) 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. ELN2017 risk stratification predicted poorly patients outcome although a NGS-based classification performed better.
The diagnosis of prePMF have been added (anemia, leukocytosis >11 ×10^9/L, and prePMF diagnosed according to the new 2016 WHO criteria. The revised WHO criteria for myeloid neoplasms was recently revised in 2016. The revised WHO criteria ACCORDING TO THE REVISED 2016 WHO DIAGNOSTIC CRITERIA THROMBOCYTHEMIA AND PREFIBROTIC MYELOFIBROSIS DIAGNOSED REPORT OF THE REGISTRO ITALIANO TROMBOCITEMIE BACKGROUND: The World Health Organization (WHO) classification system for myeloid neoplasms was recently revised in 2016. The revised WHO criteria unify the classification of different preleukemic, pre-BFM (prePMF) from "true" essential thrombocytopenia (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 ×10^9/L, 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 might contribute to the high level of platelet count observed in prePMF. ET and MPN did not differ in terms of leukocyte count, hemoglobin, platelet count, LDH, circulating CD34-positive cells and splenomegaly (Table 1). The 26 patients with MPN were not further considered in the analysis of disease complications and overall survival due to the low number. PrePMF patients had lower overall survival (overall survival at 10 years 86.4% vs 96.6%, P <.001) and a trend to a higher incidence of leukemic evolution (cumulative incidence of acute myeloid leukemia at 10 years 23.3% vs 19.0%, P =.067) compared to ET patients, while they did not differ in terms of thrombotic complications (cumulative incidence of thrombosis at 10 years 18.5% vs 18.0%, P =.86). 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 MPN 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 =.033) Summary/Conclusions: ET and prePMF diagnosed according to 2016 WHO criteria are two entities with a different clinical phenotype at diagnosis and a different outcome. The clinical phenotype at disease onset of MPN and ET is similar.

Table 1.
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 unify the classification of different preleukemic, pre-BFM (prePMF) from "true" essential thrombocytopenia (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 ×10^9/L, 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 might contribute to the high level of platelet count observed in prePMF. ET and MPN did not differ in terms of leukocyte count, hemoglobin, platelet count, LDH, circulating CD34-positive cells and splenomegaly (Table 1). The 26 patients with MPN were not further considered in the analysis of disease complications and overall survival due to the low number. PrePMF patients had lower overall survival (overall survival at 10 years 86.4% vs 96.6%, P <.001) and a trend to a higher incidence of leukemic evolution (cumulative incidence of acute myeloid leukemia at 10 years 23.3% vs 19.0%, P =.067) compared to ET patients, while they did not differ in terms of thrombotic complications (cumulative incidence of thrombosis at 10 years 18.5% vs 18.0%, P =.86). 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 MPN 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 =.033) Summary/Conclusions: ET and prePMF diagnosed according to 2016 WHO criteria are two entities with a different clinical phenotype at diagnosis and a different outcome. The clinical phenotype at disease onset of MPN and ET is similar.

Table 1.
P358

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**Background:** The minimal effective treatment in Essential Thrombocythemia (ET) patients is tailored mainly on the basis of thrombotic risk scores (primarily non-malignant). The Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) is derived from different combinations of Age >60 yrs (Age >60), JAK2 V617F mutation (JAK2+), and Prior Thrombosis (PrTh+).

**Methods:** The web-based Registro Italiano Trombocitemie (RIT) recruited since 2005 patients with thrombocytosis (bcr/abl negative chronic myeloproliferative neoplasms (MPN)). ET patients (classified according to WHO 2016 criteria) with complete information (characteristics at diagnosis, antithrombotic 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 into 4 thrombotic risk groups: Very Low Risk (VLR: Age <60, absence of JAK2 mutations, no PrTh+), Low Risk (LR: Age >60, JAK2+), Intermediate Risk (IR: Age >60, High Risk (HR: PrTh+), or Age >60 with JAK2+). The median follow-up was 12, 12, 9, and 11 years, respectively (whole cohort, 11 years).

**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%). The rates (n and%, n/100 pt-yrs) of first 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 (VLR: Age <60, absence of JAK2 mutations, no PrTh+), Low Risk (LR: Age >60, JAK2+), Intermediate 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 TFS was 0.98, 0.97, 0.84, 0.78, and 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).

**Figure 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 over-treatment seems to have occurred in this cohort of ET patients (anti-platelets in almost all cases, and cytoreduction in around 2/3 of VLR and LR cases), probably because other adjunctive risk factors have been considered.

**Background:** 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 biomarkers and baseline symptom burden, along with the potential to mitigate symptoms upon improvement of these proteins (Duck Blood, 2013). To date, no study has evaluated the correlations between elevated biomarkers (BMKs) and specific MF symptoms.

**Aims:** In this analysis of the phase-III placebo controlled COMFORT-I study, we investigated the relationships between blood BMKs and individual MF symptoms at baseline and post-treatment with ruxolitinib.

**Methods:** Biomarker levels at baseline, week 4 and 24 were measured along with MF symptoms (MSAF 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 baseline 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) as covariates.

**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+TSS were statistically significantly associated with TSS over time.

**Summary/Conclusions:** Spleen-related symptoms demonstrate close association with BMKs at baseline as well as BMKs over time, after taking into account age, sex, BMI, and treatment. At baseline and over time, abdominal symptoms remained the most frequently correlated or associated symptoms with biomarker levels. More research is needed to determine the role of some of these key BMKs in altered cellular signaling and potentially targetable pathways for symptom reduction.

Table 1.

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<th>Symptoms+TSS</th>
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<td>APOA1</td>
<td>Night sweats, itchiness, bone or muscle pain</td>
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<tr>
<td>EPO</td>
<td>Feeling full, pain under left ribs</td>
<td>20</td>
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<td>FERRITIN</td>
<td>Spleen-related symptoms</td>
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<td>MIP1A</td>
<td>Abdominal discomfort</td>
<td>23</td>
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<td>PSAF</td>
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**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+).
P360

NOVEL HETEROZYGOUS ITGB3 P.T746DEL MUTATION INDUCING SPONTANEOUS ACTIVATION OF INTEGRIN αIIbβ3 CAUSES AUTOSOMAL DOMINANT MACROTHROMBOCYTOPENIA WITH ABNORMAL αIIbβ3 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 was evaluated by flow cytometry (FCM) and western blotting (WB). The effects of mutations on αIIbβ3 expression 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 leucocyte inclusion bodies, normal ristocetin cofactor activity, 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. Immuno-fluorescence staining using CHO showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIbβ3 (p.T746del) expressing cells (Figure 1). Immunofluorescence of transfected cells showed 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) decreased these abnormalities and rendered 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.

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 uncouples 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 ITPc 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 ETLROMBOPAG (ETP)

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Background: Etrtombogap (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 (ITPc) patients.

Methods: ETPc 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 ≤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 ITPc 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: Advantia Bio’s PathwayGuide (http://www.advantibio.com/pathwayguide) and DAVID Bioinformatics Resources.

Results: The median age of the 14 ITPc 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, 15x10^9/mm^3 and 6,85x10^9/mm^3 vs P and WBC post: 132x10^9/mm^3 and 9,1x10^9/mm^3). All but two patients achieved CR (95,7%) 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).

Summary/Conclusions: In ITPc 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.

P362

DEFECTIVE PTEN REGULATION CONTRIBUTES TO B CELL HYPERRE- SPONSIVENESS IN CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is a complex autoimmune disease characterized by low platelet counts. The autoantibodies produced by autoreactive B cells against self-antigens, specifically immunoglobulin G (IgG)
antibodies against glycoprotein Iib (GP Ib/IIa) and/or GP Ib/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/IL-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 increased 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 patients.

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 increased 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). Peripheral 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 IL-2, IL-21, CD40L or anti-IgM alone or in combination for 72 h 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 mRNA was also decreased in ITP B cells. 2. The level of PTEN in B cells was slightly correlated with blood platelet count (p=0.008) and also directly correlated with the pre-B cell receptor (BCR). The phosphatase and tensin homolog (PTEN) in B cells was down-regulated, and PTEN expression 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. 4. These immature B cells in CITP patients had a greater expression of CD95 but less PTEN compared to HC suggesting that down-regulation of PTEN was associated with an increased B cell activity, but the role of PTEN in human ITP has remained unclear.

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 proplatelet formation (PPF) (Fr J Haematol. 2014;165:854-64). However, the pathogenesis of the impaired PPF in ITP is not entirely understood. Additionally, the lipid mediator sphingosine 1-phosphate (S1P) plays a critical role in megakaryocytic PPF in the bone marrow (BM) niche (J Exp Med. 2012;210:2137-40). It has been demonstrated that cell-surface S1P receptors (S1PR) on MKs trigger the activation of Gi/Rac GTPase signaling. Sphingosine kinase 2 (Sphk2) is the major isoform regulating intracellular synthesis of S1P in MKs. Additionally, intracellular S1P influences the expression of Src family kinase (SFK) in MKs, including 6 members (Fyn, Lyn, Src, Yes, Fgr, and Hck) which mutually regulate each other with Rac GTPase.

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 HSCs from the BM, (FASEB J. 2010;24:4701-10). 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 measured by ELISA. The concentration of intracellular S1P was measured using ultrahigh-performance liquid chromatography mass spectrometry (LC/MS) analysis. Intracellular Sphk2, SFKs and cell-surface S1PR were measured 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 pull-down assay.

Results: Significantly fewer numbers of proplatelet-forming MKs were observed in ITP cultures. The concentration of S1P in the plasma and 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 S1PR4 was observed in ITP MKs. We found that downstream Gi/Rac GTPase signaling 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 less Sphk2 was primarily localized to the granules of ITP MKs. Intracellular S1P of ITP MKs was further explored showing a decrease of megakaryocytic S1P production ascribed to significantly decreased Rac GTPase activity and with the overall mRNA expression of Rac and Rac1s which mutually regulate each other with Rac GTPase.

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 Gi/Rac GTPase activity in ITP-MKs. Therefore, abnormal S1P/S1P4 possibly plays a role in the pathogenesis of impaired PPF in ITP, which may be therapeutically regulated by ATRA.

P364

ANTIBODY MEDIATED GLYCAN MODIFICATION: A POTENTIAL ROLE IN PLATELET DESTRUCTION IN AUTOIMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is a bleeding disease caused by autoantibodies (AAbs) directed against platelet (PLT) glycoproteins (GP). A novel mechanism of antibody-mediated PLT destruction based on Fc-independent PLT clearance via Ashwell-Morell receptors (AMRs), which recognize glycan modifications on the surface of PLTs, has been suggested.

Aims: In this study we investigated the effects of human AAbs from ITP patients on the glycan pattern of human PLTs and the consequent impact on their survival in vivo.

Methods: Monoclonal platelet antigen capture assay (MAIPA) and lectin binding assays using normal sera from ITP patients and healthy donors were used to identify the modified sera from ITP patients and healthy donors. In LBA, after incubation with sera and PLTs from healthy donors, the modification in glycan pattern was investigated by flow cytometry using lectins; Ricinus communis agglutinin (RCA), Erythrina cristagalli lectin (ECL) and Peanut agglu-
tinin (PNA) that bind to galactose, N-acetyllactosamine and N-acetylgalactosamine 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 PNAs analyzed, and the serum level of thrombopoietin was normal or moderately increased. No specific morphological alteration of platelets was detected. Most sera 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% - 29%, range 22-40%).

**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. Antbody-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 (run-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 Sanger sequencing 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 heterozygous 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 novel variants detected with a moderate reduction of GPIa-IIa was detected, regardless of genotype at the ITGA2 locus. A defective aggregation was detected after platelet stimulation with collagen 4 mcg/ml and ADP 2 mM in the five patients investigated; normal responses were obtained using collagen 20 mcg/ml, ADP 20 mM and ristocetin 1.5 mg/mL, suggesting mild functional platelets defects. Of note, three pedigrees 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-LABEL, 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 <30×10⁹/L. Weekly SC dosing started at 1 μg/kg and was titrated in 1 μg/kg increments up to 10 μg/kg to maintain plt counts of 50-200×10⁹/L. The primary endpoint was the % of time in the first 6 months with a plt response (plt count ≥50×10⁹/L without rescue medication use in the past 4 weeks).

**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)×10⁹/L. The median (Q1, Q3)% of time with a plt response in the past 4 weeks.

**Figure 1.**

**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)×10⁹/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 platelet counts ≥2x10^9/L above baseline was 60% (25%, 84%). The median dose increased to 10 µg/kg by week 32. Median (min-max) treatment duration to date was 25 (1-67) weeks for a total exposure to date of 79 patient-years. Median (min-max) average weekly romiplostim dose 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), petechiae (n=2), decreased platelet 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), petechiae (n=2), and 1 case each of hematemesis, hematomata, SC hemorrhage, injection site hemorrhage, and mouth hemorrhage. No grade 4 or 5 bleeding was observed. No neutralizing antibodies against romiplostim 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 TIP. 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 romiplostim 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 romiplostim dose reached 10 µg/kg and there were no new safety signals. No effects of romiplostim 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 romiplostim in children with ITP of 79 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?

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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 thienopyridine 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.

Aims: The aim of this study was to assess the efficacy of six novel thienopyridine derivatives synthesized by our group as potential inhibitors of platelet function.

Methods: Healthy human platelets were isolated and incubated with novel thienopyridine compounds (DJ0081, DJ0199, DJ0206, DJ0171, DJ0021, DJ0171, DJ0097) (10µM, 30min) prior to stimulation with ADP (10µM) and analysis of alpha granule secretion (CD62P expression), GPIIb/IIIa activation (PAI1 expression) and platelet leukocyte aggregate (PLA) formation using flow cytometry. Furthermore, light transmission aggregometry (LTA) was used to assess ADP-mediated 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 experiments were performed in a total volume of 100 µl. In vitro studies included incubation of platelets with the compounds for 30 min, followed by addition of ADP (10µM), with collection of aliquots at 0, 5, 10, 15, 20, and 30 min. 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 thienopyridine compounds as modulators of platelet function and points to the possibility of future use in patients at risk of platelet hyperactivity and thrombosis.
**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 ageing 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 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 pulmonar 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 Detect D-dimer, Instrumentation Laboratory) than 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 year-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 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% of total costs (45,023.4 vs 48,356.4 Euros) for PE diagnosis and 5.1% (9,099 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 correlativey 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 levels 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.

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**Figure 1. Survival according to treatment group.**

**Summary/Conclusions:** Relapse following aHSCT 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.

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**P372**

**ACUTE MYELOID LEUKEMIA TREATMENT PRACTICE PATTERNS, HEALTHCARE RESOURCE UTILIZATION (HRU) AND COSTS IN A US COMMERCIALLY-INSURED POPULATION**

M. Hagwara1, A. Sharma1, K. Chung2, T. E. Delea1

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**Background:** AML is a rapidly progressive hematologic malignancy that accounts for 25% of all leukemias in the Western world, with 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: first 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. Pts 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 6,862 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 ($83,274). 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>UnTreated Pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCT/BCS thresholds / percent threshold</td>
<td>103</td>
<td>75</td>
</tr>
<tr>
<td>Office of non-eligible visits, times</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Outpatient pharmacy drugs, month</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Emergency department visits, times</td>
<td>1.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Hemoglobin, mean</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Hematocrit, mean</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Creatinine, mean</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Total costs, $</td>
<td>198,070</td>
<td>79,569</td>
</tr>
<tr>
<td>SCT+</td>
<td>54,236</td>
<td>19,089</td>
</tr>
<tr>
<td>SCT+</td>
<td>10,897</td>
<td>1,047</td>
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<tr>
<td>SCT+</td>
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<td>19,440</td>
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<tr>
<td>SCT+</td>
<td>8,258</td>
<td>4,236</td>
</tr>
</tbody>
</table>

Summary/Conclusions: AL amyloidosis may be greater for those with PN involvement.

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 senso-motor 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 health-related quality-of-life (HRQoL) domains: 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 ≥6 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 versus non-AL-PN patients (68.3 vs 28.8%, p <0.001). There were greater odds of experiencing numbness (OR=4.23, 95% CI: 2.45–7.30, p <0.001) and fatigue (OR=3.09, 95% CI: 1.36–7.02, p <0.01) among AL-PN patients as compared to non-AL-PN patients, even after controlling for other types of organ involvement. Similar findings were observed for gastrointestinal symptoms, such as alternating bouts of constipation or diarrhea (OR=1.92, 95% CI: 1.12–3.34, p=0.019) and early satiety/feeling fullness in the stomach (OR=1.80, 95% CI: 1.03–3.16, p=0.04). With the exception of RE, MH, and MCS, there were significant differences in SF-36v2 scores among 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 HROQL. 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 services 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 diagnosis, 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.

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. Intravenous immunoglobulin infusions, taking between 1-2 hours, were also administered. There was a consultant review clinic on board for patients receiving bortezomib which further reduced the numbers of hospital visits for patients and also a nurse-led Quality Assurance function.

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% are 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. 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.

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. Methodology: A state transition model was developed to estimate the ELN switching 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 MR², §. Duration of first-line or second-line TFR was based on an extrapolation of ENERTnd, considering treatment failure 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 MR-3/4 (163 vs 124); and 0-go (high 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 “go-on” (high probability to develop toxicity regardless of intensive or attenuated therapy). 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 budget 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
S. Bonanà1, A. Gonzalez2, A. Cruz-González3, L. García-Iglesias4, I. Jauregui5, E. Pérez-Persona5, R. Lluch6, C. Marrero7, M. Zudaire8, M. Gironella9, E. Pérez-Persona5, R. Lluch6, C. Marrero7, M. Zudaire8, M. Gironella9, Á. Ramírez-Payer10, M. Arnán11, C. Olivier12, C. Encinas13, J.A. Soler14, P. Fernández16, D. Vilanova16, J. de la Rubia17,*
1Hematology and Oncology, Mayo Clinic, Scottsdale, 2Hematology and Oncology, Oregon Health and Science University, Portland, 3Internal Medicine, 4Bio-statistics, Mayo Clinic, Scottsdale, 5Arizona State University, Phoenix, 6Hematology and Oncology, University of California, Irvine, Irvine, United States

**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 obesity (caloric deficiency). The ENERTnd registry showed that 30% (15.4% vs 14.6%) of patients that developed toxicities for each group was 34.6%, 56.3%, and 78.3% respectively with a statistically significant difference (P=0.002).

**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 find out 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 “go-on” (high probability to develop toxicity regardless of intensive or attenuated therapy). 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 budget 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.

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**Summary/Conclusions:** The GAH scale appears to have the potential to give guidance for election of individual treatment regimens. By identifying elderly patients at high risk to develop toxicities, it may help to choose low-toxicity combinations, to avoid harmful therapies and to identify those patients that could benefit from more intensive treatment. Nonetheless prospective studies with larger populations should be performed to confirm these findings and to try to determine particular cut-off points for different diseases.

P377
NUTRITIONAL NEEDS AND PREFERENCES OF MYELOPROLIFERATIVE NEOPLASM PATIENTS: PHASE IA OF THE NUTRITIONAL STUDY
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1Hematology and Oncology, Mayo Clinic, Scottsdale, 2Hematology and Oncology, Oregon Health and Science University, Portland, 3Internal Medicine, 4Bio-statistics, Mayo Clinic, Scottsdale, 5Arizona State University, Phoenix, 6Hematology and Oncology, University of California, Irvine, Irvine, United States

**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 obesity (caloric deficiency). The ENERTnd registry showed that 30% (15.4% vs 14.6%) of patients that developed toxicities for each group was 34.6%, 56.3%, and 78.3% respectively with a statistically significant difference (P=0.002).

**Aims:** To estimate the budget impact for first-line nilotinib vs imatinib when considering generic imatinib pricing, early treatment-switching, and TFR. Methodology: A state transition model was developed to estimate the ELN switching 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 MR², §. Duration of first-line or second-line TFR was based on an extrapolation of ENERTnd, considering treatment failure 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.

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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.

<table>
<thead>
<tr>
<th>Food Allergies and Intolerance</th>
<th>Frequency Among All Respondents</th>
<th>Frequency Among Those With MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>8.3%</td>
<td>9.1%</td>
</tr>
<tr>
<td>Wheat</td>
<td>6.2%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Yeast</td>
<td>4.3%</td>
<td>5.2%</td>
</tr>
<tr>
<td>Peanuts</td>
<td>4.3%</td>
<td>5.2%</td>
</tr>
<tr>
<td>Soy</td>
<td>3.1%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Tree nuts</td>
<td>2.0%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Fish</td>
<td>1.8%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Egg</td>
<td>1.2%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Cereal</td>
<td>1.2%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Nutritional supplements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>8.2%</td>
<td>9.1%</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>6.3%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>4.3%</td>
<td>5.2%</td>
</tr>
<tr>
<td>Curcumin</td>
<td>3.1%</td>
<td>3.9%</td>
</tr>
<tr>
<td>CoQ10</td>
<td>1.8%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Lactose intolerance</td>
<td>1.2%</td>
<td>1.3%</td>
</tr>
</tbody>
</table>

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.

P378

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.
Stem cell transplantation - Clinical 1

P379

OUTCOME OF ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPANTATION 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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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 allogeneic transplantation (HCT) because of concerns about increased transplantation-related toxicity and excessive and non-relapse mortality (NRM), a challenging problem especially in older individuals. However the development of reduced-intensity 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 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 separately 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 (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 disease (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 vs 29%, p<0.001), received more often reduced intensity conditioning (80 vs 63%, p<0.001). Incidence of acute GVHD grade II/III/IV, chronic GVHD and relapse were the same in the two groups in multivariate analyses. Non-relapse mortality (NRM) at 3 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.

P380

BLOOD BAALC AND MN1 COPY NUMBER ASSESSMENT BY DIGITAL DROPLET PCR PRIOR TO ALLOGENEIC TRANSPLANTATION PREDICTS RELAPSE IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Acute myeloid leukemia (AML) patients (pts) that relapse after allogeneic stem cell transplantation (H SCT) have a dismal prognosis. Identification of pts at high risk of relapse may allow preemptive therapy & improve allogeneic stem cell transplantation (HSCT) have a dismal prognosis. Identifi-

Aims: Determine the prognostic impact of peripheral blood (PB) pre-HSCT BAALC & MN1 copy numbers in an expanded set of AML pts in hematologic CR using digital droplet (dd) PCR.

Methods: We identified 118 AML pts (median age at HSCT 64 [range 31-76] years [y]) in first (55%) or second complete remission (CR; 23%) or CR with incomplete recovery (22%) with PB prior to HSCT (median 7, range 0-29 days) available. All pts received non-myeloablative (NMA) conditioning (fludarabine 3x30 mg & 200 cGy total body irradiation). At diagnosis karyotypes & NPM1, CEBPA gene mutations (mut) & presence of FLT3-TKD & FLT3-ITD were assessed. Quantification of BAALC & MN1 normalized to ABL1 copy numbers in pre-HSCT PB of the AML pts & in PB of healthy controls (n=7, median age 63 [range 40-82]) was performed by ddPCR. Median follow up after HSCT for pts alive was 1.8y.

Results: European LeukemiaNet (ELN) 2010 classification was 20% favorable, 25% intermediate-1, 24% intermediate-II, 31% adverse. AML pts & healthy controls did not differ in age (P=1) or mean BAALC (P=0.37, Figure 1A) or MN1 (P=0.96, Figure 1B) copy numbers. BAALC & MN1 copy numbers correlated well in pts (R=80) & healthy controls (R=75). The previously determined cut-off of 0.14 BAALC copy numbers (in 82 pts; ASH 2016, #517) defined pts with high (27%) & low (73%) pre-HSCT BAALC copy numbers. A cut-off of 0.74 MN1 copy numbers was determined using the R package "OptimalCutpoints" & defined pts with high (12%) & low (88%) pre-HSCT MN1 copy numbers. Applying these cut-offs, 71% of the pts had low BAALC & MN1 copy numbers & 10% had high BAALC & MN1 copy numbers, 2% had high MN1 but low BAALC & 17% had high BAALC but low MN1 copy numbers. Pts with high & low pre-HSCT MN1 copy numbers did not differ significantly in pre-treatment characteristics or remission status at HSCT (CR vs CR) while pts with high pre-HSCT BAALC copy numbers were less often in CR at HSCT (P=0.02). Both high pre-HSCT BAALC & MN1 copy numbers significantly associated with higher CIR (P<0.02, Figure 1C & P<0.01, Figure 1D, respectively). In multivariate analyses, high pre-HSCT BAALC (Hazard Ratio [HR] 2.5, Confidence Interval [CI] 1.1-5.7, P<0.001) & high pre-HSCT MN1 copy numbers (HR 5.6, CI 2.6-12.2, P<0.001) retained their prognostic impact on CIR after adjustment for ELN 2010 genetic risk groups.

Figure 1.

Summary/Conclusions: High pre-HSCT copy numbers of BAALC & MN1 associated with higher CIR in univariate & multivariate models and might indi- cate residual disease burden in these AML pts. High copy number pts should be closely monitored for relapse in the post-transplant period. Prospective clinical trials are needed to validate the determined cut-offs, to evaluate if BAALC or MN1 copy numbers or a combination of the genes represents the most suit- able prognostic signature pre-HSCT and whether AML pts with high pre-HSCT BAALC or MN1 copy numbers benefit from additional pre- or post-HSCT treat- ment.

P381

THE USE OF BPX-501 DONOR T CELL INFUSION WITH INFUNDABLE CAPSASE 9 SUCIDE GENE TOGETHER WITH HLA-HAPLOIDENTICAL STEM CELL TRANSPLANT TO TREAT CHILDREN WITH HEMOGLO- BINOPATHIES AND ERYTHROID DISORDERS

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Background: Allogeneic HSCT from either an HLA-identical sibling or an unre- lated donor is a potentially curative treatment for patients with hemoglo-

Aims: Test the use of a genetically modified donor T cell infusion therapy to treat children with hemoglobinopathies and erythroid disorders (ED), such as Thalassemia Major (TM),…
Sickle Cell Disease (SCD) and Diamond-Blackfan Anemia (DBA). Bertain et al (Blood, 2014) have previously shown that αβTCR 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 iCD9 suicide gene (BPX-501 cells). (ClinicalTrials.gov identifier: NCT02065869). The iCD9 vector contains the sequence for the CD19 marker, so that the BPX-501 cells (CD5+/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 infusing 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 ββ/β 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 chimerism. The patients with secondary graft failure were re-transplanted from the same donor and maintained full donor chimerism.

**Results:** All patients are alive and well with no Treatment Related Mortality (TRM). Initial engraftment occurred at a median of 23.5 days (range 14-55) and there were two patients re-hospitalized at 30, 163 days respectively. Grade I/II skin acute GVHD occurred in four patients and one patient had acute skin GVHD Grade IV. No chronic GVHD was observed. Median time to neutrophil recovery was 14 days (range 10-32), while 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 achieved with normal cellular and humoral immunity present at 180 days post HSCT. All patients remain transfusion-free with a median hemoglobin of 11 or greater after 6 months.

**Background:** All clinical data published thus far on the use of MSCs were generated using cells expanded from individual bone marrow donors hence suffer from huge inter-donor differences in MSC generation, expansion and immunomodulatory potential. To control these variables and to be able to administer 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 near identical phenotype and in-vitro immunomodulatory 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 allo-antigen-driven reaction in mixed lymphocyte reactions (Kuci et al. Haematologica 2016: 101 (8): 885-954).

**Aims:** A “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 severe with GVHD treated who were either non responsive to steroid or resistant to steroid treatment who failed steroid therapy before haplo-HSCT.

**Methods:** Using these standardize 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 8 years (range: 0.5-55.2). Six patients received HSCT from the same donor and maintained full donor chimerism. The patients with secondary graft failure were re-transplanted from which end-of-passage-2 MSC products are expanded for clinical use. The 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 near identical phenotype and in-vitro immunomodulatory 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 allo-antigen-driven reaction in mixed lymphocyte reactions (Kuci et al. Haematologica 2016: 101 (8): 885-954).

**Results:** Response was defined as either complete response (CR) or partial response (PR). CR were patients who showed one overall GVHD grade less according to the Glucksberg criteria, or non response (NR) on 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. The 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 OS survival probability of 72±8%.

**Summary/Conclusions:** Our Phase II/II trial with standardized equipment MSCs from the “FRANKFURT MSC-BANK” offers an excellent chance to overcome treatment-resistant and steroid-refractory acute GVHD.
and pts with unavailable TAC concentration data were excluded. A total of 253 pts were 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 methylprednisolone on day 1, 3, 6 at dose of 10 mg/m², 7mg/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)), IIP: 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=0.035), standard acute GVHD (HR: 0.44, 95%CI, 0.29-0.63), and pts with unavailable TAC concentration data were associated with poor OS. By Cox proportional-hazards regression models, TRC-EC diagnosis at day 1 was 1.90, 95%CI: 1.16-3.11, P=0.011 and high disease risk at transplant (HR: 1.76, 95%CI: 1.1-2.73, P=0.011) was significantly associated with poor OS (Figure 1).

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-REPLETATE HAPLO-IDENTICAL 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: Patients with aggressive adult T cell leukemia-lymphoma (ATL) are included in the high-risk group. The role of chemotherapy as well as the impact of reduced intensity conditioning regimen (RIC) has been conducted due to a rarity of patients with ATL even in Japan. The current study evaluated the impact of RIC on outcomes and validated treatment strategy for ATL.

Aims: The present study compared the impact of RIC and MAC in pts with AML older than 45 yrs undergoing haplo-SCT. 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 important 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 autologous 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<0.01), 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 TBF (56%), while in RIC it was miniTBF (27%) and low dose TBI+Fludarabine (24%). RIC recipients had a more frequent conditioning regimen associated with an active disease as 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% vs 0.15, GRFS 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 followed 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 (active disease vs CR): RI (HR: 2.44, p<0.005), LFS (HR: 1.75, p<0.10), GRFS (HR: 1.72, p=0.10), OS (HR: 1.71, p<0.10), 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 patients with advanced presentation. 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 designed randomized study comparing 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 in a similar decision analysis study of patients with acute myeloid leukemia were used. 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 99.8% 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.

**P386**

OUTCOMES OF THIOTEPA BASED REDUCED-INTENSITY CONDITIONING VERSUS STANDARD REDUCED-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 thiopeta and higher dose of TBI to standard RIC has shown to result in sustained donor engraftment. We 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 (200cGy or 300cGy) versus this standard-RIC regimen with addition of thiopeta (10mg/kg) and increased dose of TBI (50cGy).

**Aims:** To compare transplant related outcomes in CBT recipients who received standard-RIC (FluCyTBI) to those who received novel-RIC (FluCy with addition of thiopeta and increased dose of TBI). 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:** of 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, 0-6) 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 (40.4%) 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-RIC cohort was 9.3 months (range, 0.16-79) and 13 months (range, 1.4-36) in novel-RIC cohort. The overall survival (OS) was significantly better in novel-RIC cohort compared to standard-RIC (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).

**Figure 1.**

**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.

**P387**

INTERFERON-A IS EFFECTIVE FOR TREATMENT OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH ACUTE LEUKEMIA 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-α (IFN-α) 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 (cGVHD) in the AZA and BSC-treated groups was 10% and 22%, respectively (AZA vs. BSC, P=0.66), relapse (31.5% and 28.6%, P=0.59), NRM (26.5% and 26.1%, P=0.98), and PFS (70.8% and 70.0%, P=0.66) were not significantly different between the AZA and BSC groups. In multivariate analysis, AZA and BSC were not independently associated with the occurrence of relapse, non-relapse mortality (NRM), and hematopoietic recovery after allo-HSCT.

Conclusions: Our study suggested that pretransplant induction therapy in high-risk MDS patients. However, the benefits of pretransplantation 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 Japanese 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 underwent their first transplantation between January 2009 and December 2014 and received AZA or BSC as 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 between AZA and BSC patients who were pretreated with AZA.

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 replaced cord blood transplantation (72.0% and 69.4%, P=0.36) were not significantly different between the AZA and BSC groups. In multivariate 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 genotypic matching, and conditioning regimen.

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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. 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 differentiations were also confirmed using Western blotting.

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 < 100×10⁹/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/m², intravenously daily for 4-6 weeks).

Results: Major response was observed in 16 out of 19 patients (84.2%) in the 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, which is consistent with previously reported results. Further studies are needed to establish whether the additional administration of decitabine to patients with HSCT-related thrombocytopenia can improve platelet recovery.
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-eIF2a, indicating 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 THALASSEMIA PHENOTYPE
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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 and the fetal-to-adult globin gene switching (Waye JS et al Int. J. Lab. Hem. 2015; 37: 78-84). It has been reported that mutations leading to KLF1 haploinsufficiency are linked to increased fetal hemoglobin (HbF) levels with amelioration of the condition of a neutral polymorphism. Furthermore, unexpectedly, the novel F182L and M39L mutations were found to be associated with increased fetal- and/or beta-globin gene expression. In other cases we demonstrated that KLF1 mutations may contribute to worsen the beta thalassemia phenotype or result in a silent beta thalassemia trait. This study provides further insights into the multiple roles of KLF1 in erythropoiesis and highlights an intriguing effect of a subset of KLF1 mutations that may contribute to the severity of the thalassemia phenotype, thus reinforcing the relevant implications of KLF1 screening for genetic counseling and for effectiveness of prevention screening programs for hemoglobinopathies.

RESULTS: Of the 19 patients who were tested, 15 were found to be positive for mutations in the KLF1 gene. More in detail, we found 7 mutations, comprising a nucleotide variation (c.-251 C>G) already reported as a single nucleotide polymorphism and a known mutation (c.-148 G>A) in the proximal promoter region, 3 novel mutations (C94X and P173Pfs*236), all of them in the proline-rich domain of the gene, and 8 subjects with nonsynonymous mutations (S102P, F182L, and M39L), which were corroborated by in silico prediction tools (S102P, F182L, M39L, (Radmilovic M. et al. Ann. Hematol 2013; 92: 53-58) and 2 novel mutations (C94X and P173Pfs*236), all of them in the proline-rich domain in exon 2. Functional studies were performed in K562 cells in order to clarify the pathogenic significance of these mutations and to better define the role of KLF1 in atypical thalassemia phenotypes. Interestingly, the c.-251 C>G polymorphism was found to be associated with an increased transcriptional activity of the KLF1 promoter (Figure 1A), thus allowing us to exclude for this nucleotide variation the condition of a neutral polymorphism. Furthermore, unexpectedly, the novel P173Pfs*236 mutation was found to be associated with a dramatic reduction of the beta-globin gene expression (Figure 1B).

Summary/Conclusions: Our study confirmed the ameliorative effect of some KLF1 mutations on the thalassemia phenotype that were found to be associated with increased fetal- and/or beta-globin gene expression. In other cases we demonstrated that KLF1 mutations may contribute to worsen the beta thalassemia phenotype or result in a silent beta thalassemia trait. This study provides further insights into the multiple roles of KLF1 in erythropoiesis and highlights an intriguing effect of a subset of KLF1 mutations that may contribute to the severity of the thalassemia phenotype, thus reinforcing the relevant implications of KLF1 screening for genetic counseling and for effectiveness of prevention screening programs for hemoglobinopathies.

P394
SECONDARY SOLID TUMORS FOLLOWING HEMATOPOietic CELL TRANSPLANTATION FOR THALASSEMIA MAJOR
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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 treated with TM who received 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 malignancy in 64 months (median age 10 years at time of marrow donation) after HCT including 1 carcinoma of the tongue, 1 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 4 of them received chemotherapy and/or radiotherapy. Of relevance, the 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 5 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±0.2% with a statistically significant difference (p=0.03) as compared to that of transplanted patients. The second case control population consisted of 117 patients treated with HCT and affected by SST. The matching technique applied was based on the variables age and sex. One control per case (transplanted patient) was randomly selected from the MIOT (Myocardial Iron Overload in Thalassemia) registry and matched by sex and age with the transplanted patient population. Two patients developed an hematocellular carcinoma (HCC) at age of 39 and 44 years, respectively. One patient died and one is living. Using the event rate measure, we observed an event rate of 0.102 at 30 years for the transplant group and 0.041 for the nontransplant group (p=0.106).

Summary/Conclusions: This study shows that the magnitude of increased risk of SST is twofold to threefold for patients treated with HCT as compared with age- and sex matched nontransplant TM patients or with 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 careful monitoring and surveillance in order to prevent the development of secondary cancers.
Background: Newborn screening program for thalassemia (thal) and hemoglobinopathies (NHS-Hbs) is crucial for early detecting patients with serious hemoglobinopathies (Hb variants) e.g. Sickle cell anemia (Hb SS). NHS-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, NHS-Hbs can detect several types of thalassemia and Hb variants carriers. This application could be useful for the national prevention and control program to better control 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 NHS-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 NHS-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 ARM-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 ≥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 was identified and they had a lower level of Hb A as compared to their gestational age (GA)-sex matched 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 following by molecular analysis.

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 NHS-Hbs into a routine service in order to early detect Hb H disease, Hb E/β-thalassemia and the majority of common thalassemia carriers. This NHS-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 NHS programs currently available in several European countries.

P397

INCREASING INCIDENCE OF MALIGNANCIES IN AGING THALASSEMIC PATIENTS: A SINGLE INSTITUTION'S LONGITUDINAL EXPERIENCE

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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 experience some of the 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 thalassemic 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 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. were withdrawn from the study and placed on a standard chelation regi-
was assessed at 6, 9 and 12 months (patients confirmed SF ≥1000 ng/mL
patients with LPI ≥0.6 µM, SF ≥1000 μg/L or TSAT ≥70% in each study arm
dose (50 mg/kg/day) or no chelation (NC). Median age at 1st transfusion
≥0.2 µM were randomized to start deferiprone (DFP) at a sub-therapeutic
≥400 μg/L or transferrin saturation (TSAT) ≥70% or labile plasma iron (LPI)
Summary/Conclusions: This retrospective study has confirmed the increased
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,
thoracic and hematologic, will allow early detection and timely, and thus, more
efficacious treatment of the neoplasia.

SAFETY AND EFFICACY OF EARLY START WITH SUBOPTIMAL DOSE
OF DEFERIPRONE IN MINIMALLY TRANSFUSED INFANTS WITH
TRANSFUSION DEPENDENT THALASSEMIA: A RANDOMIZED TRIAL
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Background: Early exposure to iron toxicity is the main risk factor for morbidity and mortality
in patients with transfusion-dependent thalassemia. Current prac-
tice is to start chelation therapy only after 10-20 transfusions, or when the
serum ferritin (SF) level rises above 1,000 µg/L.

Aims: To evaluate the safety and efficacy of the early use of low-dose deferiprone in minimally transfused pediatric thalassemia patients and to evaluate
if it can postpone iron overload in these group of patients.

Methods: In the current trial (ClinicalTrials.gov Identifier: NCT02173951),
64 children recently diagnosed with thalassemia major who had
begun receiving blood transfusions in first year of life to keep pre-transfusion
Hb above 10 g/dl, had not yet started iron chelation therapy and had SF
≥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). Median age at 1st transfusion
was 5 months for both DFP-treated and for NC children. The percentage of patients with
LPI ≥0.6 µM, SF ≥1000 µg/L or TSAT ≥70% in each study arm
was assessed at 6, 9 and 12 months (patients confirmed SF ≥1000 ng/mL
were withdrawn from the study and placed on a standard chelation regi-
men). Complete blood count was done weekly in DFP treated and every 3-
week intervals in NC.

Results: Table 1. Summary of the efficacy results of SF, TSAT, and LPI.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
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<tbody>
<tr>
<td>SF (µg/L)</td>
<td>43 ± 25</td>
<td>69 ± 37</td>
<td>60 ± 30</td>
<td>55 ± 26</td>
<td>50 ± 22</td>
</tr>
<tr>
<td>% patients with SF ≥1000 µg/L</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>TSAT (µ%)</td>
<td>40 ± 9</td>
<td>44 ± 11</td>
<td>48 ± 13</td>
<td>52 ± 15</td>
<td>56 ± 17</td>
</tr>
<tr>
<td>% patients with TSAT ≥70%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>LPI (µM)</td>
<td>0.28 ± 0.02</td>
<td>0.46 ± 0.12</td>
<td>0.54 ± 0.25</td>
<td>0.68 ± 0.35</td>
<td>0.82 ± 0.50</td>
</tr>
<tr>
<td>% patients with LPI ≥0.6 µM</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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</tr>
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</table>

All NC patients were removed from the trial prior to completing 7 months of follow-
up (9-11 transfusions ) due to confirmed SF ≥1000 µg/L. Mean ± SD time of follow up
was 10.4 ± 4.9 and 5.9 ± 2.5 months for DFP and NC respectively.

Most common adverse events in patients on DFP versus NC were diarrhea
(19% vs 13%, p= 0.73), vomiting (13% vs 13%, p=1.00), abdominal colic (13% vs
13%), elevated liver enzymes (6% vs 3%, p=1.00) and neutropenia (6% vs
6%). All adverse events were mild in severity and did not require interruption
of DFP use. There were no cases of agranulocytosis or moderate neutropenia,
no arthralgia and no serious infections in DFP-treated patients. DFP therapy
was associated with a significant reduction in the rate of iron accumulation as
measured by SF (P<0.0001), LPI (P<0.001) and TSAT (P<0.001) (Figure 1a, b, c).
LPI ≥0.6 µM appeared as early as after 5 transfusions in NC children and
was delayed to at least 10 transfusions with DFP therapy. TSAT ≥70% appeared
after 10 transfusions in NC children and was delayed to at least 17 transfusions
with DFP therapy. The results of this study show that LPI and TSAT may reach
values ≥0.6 µM and ≥70%, respectively, after 5 - 10 transfusions in children
with TM and all NC children had SF ≥1000 µg/L after 8-9 transfusions.

Summary/Conclusions: A sub-therapeutic dose of deferiprone for a mean
of 12 months in children with TM and low iron overload was not associated
with safety concerns and able to significantly reduce the rate of iron accumulation
as measured by SF, LPI and TSAT.

LONGITUDINAL PROSPECTIVE MRI STUDY IN PEDIATRIC PATIENTS
WITH THALASSEMIA MAJOR
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Background: No studies are available in literature evaluating, on repeated
magnetic resonance (MR) imaging assessments, changes in myocardial and
hepatic iron overload, biventricular function, and development of macroscopic
myocardial fibrosis in pediatric patients with thalassemia major (TM)

Aims: This is the first longitudinal prospective MRI study in pediatric TM
patients

Methods: We considered 68 TM patients enrolled in the MIOT (Myocardial
Iron Overload in Thalassemia) project with less than 18 years at the first MRI
scan and who performed a follow-up (FU) study at 18±3 months.

Myocardial and hepatic iron burdens 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: At the baseline MRI, 16 (23.5%) patients showed myocardial iron overload
(MIO; global heart T2*<20 ms) and 54 patients liver iron overload

Figure 1.
(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 presence 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.

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-THALASSEMAIA 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 Jan08-Dec16.

Results: 69 TM patients: 43% male, age 38±9yrs, 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%), deferoxamine (basal 45%>f.u.52%), daily alternating deferasirox+deferoxamine (basal 3%-f.u.6%), deferoxamine (basal 9%>f.u.6%) deferoxamine+ deferasirox (Rp=0.68, p<0.001) and heart (Rp=0.75, p<0.001), in accordance with literature. 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=0.25, p=0.04) and pancreas (Rp=0.41,p<0.001). Finally, assuming the cutoff 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.

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: A HTLV-IG product isolated from an HTLV-1 carrier with a proviral load (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-Prkdcscid Il2rtm1Sug/Jic) 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 replication assay. 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 COMBINATION OF TUMOR CELLS IN THE APERIPHERIS MATERIAL DOES NOT PREDICT THE RESPONSE 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 negativley affect responses to ASCT.

Methods: Patients (n=114) undergoing ASCT (n=120) for MM between January 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, the mobilization scheme were determined. Response was assessed at day +100 after ASCT using the 2006 International Myeloma Working Group uniform response criteria.

Results: Patient characteristics are shown in Table 1. Patients with a better ASCT response to induction therapy (complete response [CR] or very good partial response [VGPR]) had a non-significant different median A:T PC ratio compared to those with a poorer response (partial response [PR], stable [SD] or progressive disease [PD]), (0.4 vs 1, p=0.28). Similarly, a non-significant difference (p=0.251) was observed in the number of atypical PC contained in the autograft of patients with a better vs poorer prior ASCT 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 (p=0.06). 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=1.26 ×10^-4). 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=0.039-7).

Table 1.
**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. Specialities included Surgery, Anesthetics, 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 syndromes (ACS). However, a threshold of ≤80g/L was selected by 42% (58), but there was a wide spread of answers. 65% (90) of participants were aware that, in a stable patient Hb is checked after each unit of red cell transfusion, but surprisingly a few (4%, 5) did not check post 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 anaemia. 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 their indication for transfusion to patients. Also 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) experienced difficulty in obtaining a patient information leaflet, and issues relating to lack of time and information 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 higher mobile group, the survey is a constructive and supportive method to facilitate implementation of national guidance 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 working closely with the hospital transfusion team to ensure that all information is disseminated to all hospital staff, and carrying out structured case based discussion sessions with junior doctors to enhance knowledge and confidence.

**P406**

**SCREENING OF TRANSFUSION PRODUCTS FOR PRION DISEASES USING APTAMERS AND TUNABLE RESISTIVE PULSE SENSING**

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**Background:** Prion diseases are a group of fatal transmissible neurological conditions whose disease etiology is characterised by the change in the normal intrinsic cellular prion protein (PrPc) to the highly ordered insoluble amyloid state conformer (PrPSc). The significant event fundamental to the progression of these diseases is the self-catalytic, and perpetuating, nature of the conversion of PrPc in the presence of PrPSc aggregates. The disease process is slowly progressive. The first major outbreak in the United Kingdom during the 1990s, is considered to be an effect of dietary exposure to the bovine spongiform encephalopathy (BSE) agent through contaminated meat products. To date, the widely accepted hypothesis is that dietary exposure to the bovine spongiform encephalopathy (BSE) agent through contaminated meat products.

**Results:** PrPc has been shown to have a mean zeta value of -6.60 mV for 100%. The assay was further developed by monitoring the functionalized nanoparticles were then used to detect the cellular prion protein in phosphate buffered saline by monitoring the relative change in velocity through the nanopore, which is then converted to zeta potential. The method was then applied to protein rich samples and serum.

**Methods:** Here we use a technique based on the Coulter Counter principle that uses tunable elastomeric nanopores termed Tunable Resistive Pulse Sensing (TRPS) to detect the prion protein without an amplification step. The first stage optimises the grafting of an ssDNA aptamer onto nanoparticles. In proof of concept work, the functionalized nanoparticles were observed. Here mean zeta values were -1.94 mV for 0%; -4.43 mV for 33%; and -7.30 mV for 100%. The assay was further developed by monitoring the functionalized particle's translocation velocity as a function of prion protein concentration. The method was then applied to protein rich samples and serum.

**Results:** By varying the concentration of aptamer relative to the binding capacity of the nanoparticles, a significant change (p=0.05) in velocity distribution was observed. Here mean zeta values were -1.94 mV for 0%; -4.43 mV for 33%; and -7.30 mV for 100%.

**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.

**Summary/Conclusions:** A comprehensive proteomics study on platelet concentrates: platelet proteome, storage time and Mirasol pathogen reduction technology

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**Background:** Platelet concentrates (PCs) represent a blood transfusion product with a major concern for safety as their storage time (20-24°C) allows bacterial, fungal, and viral contamination, 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, PRTs (such as Mirasol technology) have been developed and are implemented in many countries. However, several studies have shown that Mirasol PRT introduces a certain level of platelet shape change, hyperactivation, basal degranulation and increased oxidative damage during storage. It has been suggested that Mirasol PRT might accelerate what has been described as the platelet storage lesion (PSL), but supportive molecular signatures have not been obtained.

**Aims:**

We aimed at dissecting the influence of both variables, i.e. Mirasol PRT and storage time, at the proteome level.

**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. In our study we used LFQ and thereby targeted differentially expressed 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 affect PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to affect PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to affect PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to affect PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to affect PCs during storage.

**Summary/Conclusions:** In summary, semi-quantitative proteomics allows to discern between treatment 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**

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**Background:** UK guidelines to provide evidence-based support for decisions to transfuse were updated red cells were published in 2015 by NICE (National Institute for Health and Care 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 anemia pathway. A local baseline audit of NICE compliance at our London tertiary referral hospital showed low overall compliance with these recommendations.

**Aims:** To determine knowledge amongst the prescriber group of transfusion guidelines for recommended stable patients, to gain insight into current patterns of decision-making for transfusion and to impart knowledge of the key NICE guidelines.

**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
QUADRUPLE 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 d1-2,6,9,14,15 (20mg/m² #1d1-2), cyclophosphamide (cyclo) 500mg PO d1, 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, len 25mg PO d1-21 PO daily, dex 40mg PO d1-4,8,9,15-16), KCRD (cyclo 500mg PO d1,15 thalidomide 100-200mg PO daily, dex 40mg PO d1-4,12,15) given to max. response. Patients with VGPR/CRC proceeded straight to ASCT, those with PR/MR were randomised to sequential CVD (cyclo 500mg d1,15, bortezomib 1.3mg/m² IVSC d1,4,8,11, dex 20mg PO d1,12,15,9,11,12) or nothing and those with SD/PD all received single-agent ixazomib maintenance.

Results: 2568 TE patients underwent induction randomisation (CTD:1021, CRD:526). Patients were comparable with respect to age (median 59 years), sex and other key laboratory parameters. Patients were mandated to receive at least a minimum of 4 cycles of initial induction with therapy continued to maximum response. The median number of cycles delivered was CTD: 5, CRD: 5, KCRD: 4, Grade ≥3 haematological toxicities differed between the groups. (Neutropenia CTD: 12%, CRD: 22%, KCRD: 16%; Thrombocytopenia CTD: 3.4%, CRD: 4.5%, KCRD: 8.1%; Anaemia CTD: 6.7%, CRD: 9.6%, KCRD 10%). Grade ≥2 neurological toxicity was greater with the thalidomide-containing regimen (Sensory neuropathy CTD: 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 (CTD: 11.8%, CRD 11.1%, 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

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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.
Methods: In this phase 1/2 study (NCT01217957), patients with NDMM received weekly oral ixazomib (1.88-3.65mg/m²; days 1, 8, 15) and dexamethasone (40mg, days 1, 8, 15, and 22) for up to 12-28 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 159 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] + complete response [CR]) was 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 3.5 years. The 3-year OS estimate 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 viral 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% VGPR and 32% CR. Responses deepened during maintenance; at data cut-off, the response rates in this maintenance therapy population were ORR 32%, PR 32%, and CR 10%. 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.
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

S411
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 AL AMYLOIDOSIS
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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 I-IV, ejection fraction >45%, were included. Major exclusion criteria were symptomatic orthostatic hypotension, NT proBNP level >5000 pg/ml, Troponin T > 0.06 u/ml, Bilirubin >2x ULN, eGFR<30 ml/min, 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/m2twice a week for 2 weeks 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 died (both amyloidosis related) and 3 miscellaneous. Of these 38 patients, 3 went subsequently off protocol because of ineligibility 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.
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 into 1 of 3 independent cohorts (Cohort A: BV-naïve; Cohort B: BV after ASCT; Cohort C: BV after ASCT and brentuximab vedotin (BV) treatment. Per Independent Radiology Review Committee. Secondary endpoints included ORR, encouraging duration of response (DOR) and an acceptable safety profile.

Methods: This 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 could resume at relapse. Primary endpoint was ORR per Independent Radiology Review Committee. Secondary endpoints included DOR, progression-free survival (PFS), overall survival (OS), and safety were exploratory endpoints. All pts provided written informed consent.

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 [n=12]) ASCT. Median (range) age was 34 (18-72) y and 77% of pts had advanced (stage III+/IV) 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 ±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 serious AEs were fatigue (23%), diarrhea (15%), infusion reactions (IRs; 14%), and rash (12%); 3-4 drug-related AEs in 33% of pts were grade 2 or greater, and alanine aminotransferase increases (3%), and neutropenia (3%). The most common drug-related serious AEs were PR (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.

References:
1A. Gallamini et al., Haematologica 2016). Durable responses to therapy are valuable in pts with progressive disease after failure of ASCT due to their limited translation to practice, efficacy results by sequencing of prior BV treatment will be presented.

Figure 1. 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 [n=12]) ASCT. 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 ±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 serious AEs were fatigue (23%), diarrhea (15%), infusion reactions (IRs; 14%), and rash (12%); 3-4 drug-related AEs in 33% of pts were grade 2 or greater, and alanine aminotransferase increases (3%), and neutropenia (3%). The most common drug-related serious AEs were PR (2%) and pneumonitis (1%). To facilitate translation to practice, efficacy results by sequencing of prior BV treatment will be presented.
Overall, 150 (19.2%) proved PET2+ (97 score 4, 53 score 5) and 630 (80.5%) PET−. PET2+ patients were more frequently male (56.7% vs 47.1%, p=0.03), had higher IPS score (P=0.0002) and bulky disease (28.0% vs 17.9%; p=0.002). Out of 149 PET2+ patients randomized to Be+Bb (76) or Be+Bb+R (73), 136 were evaluable for response: 93 obtained CR and 43 had a treatment failure. Of the remaining 13 patients, 3 died, 7 withdrew their consent and 3 stopped treatment for toxicity. As per study protocol, 627 out of 630 PET2− patients continued with 4 ABVD cycles and 3 withdrew their consent. Overall, 30 patients (3.8%) died, due to early death (n=2), resistant disease (n=18; 12 with a positive and 6 with a negative PET2), transplant related toxicity (n=5), infections (n=4) and pulmonary fibrosis (n=1). After a median follow-up of 1303 days (2-2857), the 4-Y PFS and OS for all 782 patients was 93% (95% CI 87%-96%) and 96% (95% CI 83%-98%) and 96% (95% CI 94%-97%), respectively. For PET2+ and PET2− patients, the 4-Y PFS was 69% (95% CI 60%-76%) and 87% (95% CI 84%-89%), while the 4-Y OS was 69% (95% CI 82%-83%) and 97% (95% CI 95%-98%) (Figure 1, Panel A and B). No outcome difference was observed for Be+Bb vs Be+Bb+R patients, with a 4-Y PFS of 69% (95% CI 57%-79%) and 68% (95% CI 55%-78%), respectively (p=0.9731). Consolidation RxC in PET2− patients in CR after 6 ABVD and LNM did not translate in to a significant benefit, with a 4-Y PFS of 93% (95% CI 91%-98%) for RxC and 97% (95% CI 87%-96%) for NFT (p=0.2882).

Figure 1.

Summary/Conclusions: These data suggest that 1) an early switch from ABVD to escalated BEACOPP can be safely done in PET2+ advanced-stage cHL; 2) the long-term outcome for the entire patient cohort is superior to standard ABVD; 3) no clinical benefit is associated with post ABVD RxC in PET2− patients presenting with large nodal mass; 4) the addition of Rituximab does not increase the effectiveness of Be+Bb in PET2+ patients. 

S414

DISEASE CHARACTERISTICS AND SURVIVAL AFTER 3RD RECURRENCE OF CLASSICAL HODGKIN LYMPHOMA: AN ANALYSIS OF THE GERMAN HODGKIN STUDY GROUP

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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 are typically initially 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 tumor-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 an event; 12 patients (17.1%) were censored due to competing risk. 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).

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.

S415

A REVISED STAGING SYSTEM FOR WALDENSTRÖM’S MACROGLOBULINEMIA

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Background: Waldenström’s macroglobulinemia (WM) is a rare low-grade B-cell lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and production of monoclonal IgM paraprotein. WM is an indolent lymphoma that has heterogeneous clinical manifestations and patients with this disease may have a prolonged disease course; however, there are groups of patients with poor outcomes after a relatively short disease course. In order to develop a robust staging system a collaborative effort resulted in the formulation of the International Prognostic Scoring System for WM (IPSS-WM) which was developed in 2009 based on data of patients that were treated primarily without rituximab and mainly with alkylators and nucleoside analogues. IPSSWM is based on five covariates (age, hemoglobin, platelet counts, IgM levels and b2 microglobulin) and stratifies WM patients into 3 broad risk groups. IPSSWM does not take into account non-HM used mortality, which is common and quite different among patients over the age of 75 years and does not include LDH, which is a well identified prognostic factor both in lymphomas and multiple myeloma.

Table 1.

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.
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 (>7g/dl) was quite rare and of limited prognostic value. The presence of anemia <11.5gr/dl was common across all subgroups while low platelet counts <100x10^9/L was found in relatively few patients and had no prognostic significance. Based on ROC analysis for early death (within 3 years), serum albumin <3.5gr/dl and b2microglobulin >4mg/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 0.711 (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.

Table 1.

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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.

S416

SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) TREATED WITH RITUXIMAB (R) MONOTHERAPY: A LONG TERM FOLLOW-UP STUDY ON 104 PATIENTS


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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/m^2 (induction phase). None of the pts had been splenectomised before R treatment. Maintenance with R at a dose of 375mg/m^2 every 2 months for 1-2 years was given according to physician’s discretion. Response assessment was based on the SLSG 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 SLSG 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% and 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.
Biology of MPN: JAK2 and beyond

S417

YOU DON’T KNOW JAK: A PROGRAMMED RIBOSOMAL FRAMESHIFTING DEFECT POTENTIATES THE TRANSFORMING ACTIVITY OF THE JAK2-V617F MUTATION

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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 however if 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 (Bleew AT et al, Nature, 2014). In this process, cis-acting mRNA elements (-1 PRF signals, which consist of a slippery site followed by a pseudoknot) direct translating ribosomes to slip by one base in the 3’ 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 a -1 PRF fusion protein. -1 PRF as well as mRNA abundance and decay were assayed in HEK293T and HeLa cells. Transformation assays were performed in HEK293 expressing Ba/F3 cells, in vivo experiments were performed in BALB/c mice.

Figure 1.

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 V617 (V617m) or the slippery site (SSm), both of which drastically 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 V617m 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 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 HSP-90 inhibitor, which reduce kinase activity and JAK2 expression respectively, leads to increased therapeutic efficacy in myeloproliferative neoplasms (Bhagvat N et al, Blood, 2014).

S418

EFFECTIVENESS OF LSD1 INHIBITION FOR THE TREATMENT OF MPN

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Background: Treatment of MPN with JAK inhibitors ameliorates symptoms and splenomegaly but does not meaningfully reduce the JAK2V617F allele burden. Though curative, stem cell transplantation is associated with extensive morbidity and mortality highlighting the need for novel effective therapies. The histone “lysine-specific demethylase 1A” (LSD1/KDM1A) is being explored as a drug target. JAK inhibitors alone already demonstrate durable responses which is critical for sustaining self-renewal in leukemic initiating cells; inhibiting LSD1 induces monocytic differentiation and reduces engraftment of AML cell in vivo. LSD1 is over-expressed in a number of myeloid diseases including MPN. Preliminary data in a mutant mpl mouse model of MPN showed that a 28-day course of LSD1 inhibition had a beneficial impact on spleen size, cytokines and mutant cell burden. In a mutant JAK2 mouse model of MPN, we have characterized the disease-modifying activity of the LSD1 inhibitor (IMG-7289), a compound in clinical development for myeloid disorders (NCT02842827).

Aims: We assessed the pharmacodynamic effects of continuous daily treatment with IMG-7289 in a JAK2V617F knock-in murine MPN with established disease. Animals were treated for up to 56 days. Outcome measures included complete blood counts (CBC), hematological phenotype, overall survival, spleen size, bone marrow morphology and the JAK2V617F allele burden. Moreover, pro-inflammatory cytokine were monitored during the course of treatment as well as chromatin changes by western blotting and ChIP-seq.

Methods: JAK2V617F-FL2 mice were crossed to MxCRE mice and displayed a fulminant MPN phenotype without did induction. CBC and BM FACs analysis were conducted as previously described. We designed a qPCR assay to quantify murine JAK2V617F allele burden.

Results: IMG-7289 treatment was exceptionally well tolerated and mice showed drastic decreases in platelet count (208 vs 2063*10^3/μl), reticulocytes (800 vs 1674*10^3/μl), monocytes and neutrophils 14 days after the start of treatment. HCT and WBC started to decrease after 28 days. While the JAK2 mutant allele burden increased over time in untreated mice, it decreased in IMG-7289 treated mice and drastically significantly in peripheral blood as well as in spleen. We observed a drastic increase in the pro-inflammatory cytokine CXCL5 in untreated mice during the course of investigation while CXCL5 levels of treated mice decreased to levels of wild-type littermates. Moreover, treated mice showed a highly significantly increased survival over untreated mice, even in a late stage of disease. Lastly, we were able to show that global H3K9me2, which is generally associated with gene expression silencing, was increased in the bone marrow of IMG-7289 treated mice compared to control mice. The remaining pathophysiological data and functional data on epigenetic regulation will be presented.

Summary/Conclusions: The LSD1 inhibitor IMG-7289 normalizes or stabilizes elevated CBCs in a JAK2V617F MPN mouse model. It decreases JAK2 mutant allele burden, pro-inflammatory cytokine levels and confers a clear survival advantage. Our preliminary data show that LSD1 is a potent target with disease-modifying potential in MPN. Clinical studies with IMG-7289 testing this hypothesis have just begun. Owing to its mode of action, altering epigenetics, and the potential reversibility of drug-induced epigenetic remodeling, a long treatment period in MPN patients may be necessary to eliminate disease. Combining IMG-7289 with JAK1/2 inhibitors might accelerate treatment effects.

S419

LOSS OF RAF KINASE INHIBITOR PROTEIN IS INVOLVED IN MYELOMONOCYTIC LINEAGE COMMITMENT AND AGGRAVATES THE DEVELOPMENT OF CHRONIC MYELOMONOCYTIC LEUKEMIA IN A MURINE IN-VIVO MODEL

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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 of 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 transduced with RKIP shRNA, as well as in a genetic mouse model for RKIP deletion (RKIP-−/−). Effects of RKIP on CMML development were initially studied in the same RKIP-−/− model. Additionally, these mice were crossed with animals exhibiting a somatically inducible mutation in NRAS (RKIP−/−Mx1-Cre-NRASG12D) 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−/− 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−/− mice, it aggravated the CMML-MPD phenotype in animals carrying an additional mutation in NRAS. In this case, RKIP loss caused worsening of leucocytosis (P=0.035) and splenomegaly (P=0.025), 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 (PMID: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 down-regulation of several signaling pathways, including Hedgehog, and increased inflammation associated gene expression in EZH2-KO HSCs. Unexpectedly in this mouse model of thrombocytosis, phenotypic analysis of the HSC compartment in the BM showed that platelet-biased HSCs might selectively promote development of an ET phenotype.

Methods: We generated a 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 in 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 (PMID:20489053). EZH2-KO hJAK2V617F BM recipients showed long-term serial 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 HSCs from Ezh2-KO hJAK2V617F mice primarily gave rise to platelet/myeloid lineage-committed HSCs.

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 phenotype 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/myeloid 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.
<p>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-AP/BP or Ph+ ALL (enrollment closed early due to poor response), and (3) newly diagnosed 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 of clinical interest). 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 intolerant, 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 met as early 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 pleural/pericardial effusion, pulmonary edema/hypertension, or pericardial effusion were reported here. Hypersensitivity in a newly diagnosed pt was the only DAS-related AE that led to discontinuation. Summary/Conclusions: Results from the largest prospective and registrational trial of pediatric pts with CML-CP demonstrate that DAS is a safe and effective treatment for pediatric CML-CP. Target responses for first- or second-line therapy at 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.</p>
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 ≥14a2 transcripts—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 (MMR) rate at 12 months (primary endpoint) was significantly higher with BOS versus IM in the mITT population (47.2% vs 36.9%; 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 57.3%; P<0.001); 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 only baseline characteristic identified as a significant predictor of MMR at 12 months besides treatment arm was Sokal risk group (high vs low; P=0.0001 and intermediate vs low; P=0.05 [mITT]). On-treatment progression to accelerated or blast phase occurred in 4 patients (1.6%) receiving BOS and 6 patients (2.5%) receiving IM in the mITT population. One BOS-treated and 4 IM-treated patients discontinued treatment due to progression to accelerated or blast phase. Among all treated patients, there were no deaths within 28 days of last dose of BOS and 4 with IM. Safety data for treated patients were consistent with the known safety profiles of BOS and IM. Discontinuation due to drug-related toxicity occurred with 12.7% of BOS patients and 8.7% of IM patients. Grade ≥3 diarrhea (7.8% vs 3.0%, 1.5%, and 0.4% BOS vs IM) and increased alanine (19.0% vs 3.0%, 1.5%, and 0.4% BOS vs IM) were more common with BOS. Cardiovascular, peripheral vascular, and cerebrovascular events were infrequent in both groups (all grades: 3.0%, 1.5%, and 0% BOS vs 0.4%, 1.1%, and 0.4% IM; grade ≥3: 1.5%, 0%, and 0% BOS vs 0%, 0%, and 0.4% IM).

Table 1.

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.
A ML Biology II: Epigenetic targets

S427

ETO2-GLIS2 RECRUTIS ETO2/ERG COMPLEX AT SUPER-ENHANCERS TO CONTROL TRANSCRIPTION AND DRIVE LEUKEMIC PROPERTIES IN PEDIATRIC ACUTE MEGAKARYOBLASTIC LEUKEMIA

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 ChIPseq experiments. NHR2 perinuclear localization prevented the oligomerization, reversed the transcriptional activation at enhancers, promoted megakaryocytic differentiation and abrogated human AMKL cells maintenance in vitro. So, the interaction of ETO2-GLIS2 with ETO2 complexes is an essential node for the transcriptional control by the fusion at enhancer elements. Finally, ERG is localized at super-enhancers and is involved in the 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 MYELOIDDIFFERENTIATION AND PROMOTES CLONAL DOMINANCE VIA ABRUPT HISTONE REARRANGEMENTS

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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-remodelling 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 proteomic analysis. We performed MINT-ChIP-seq (MNase 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 subpopulations 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 undifferentiated 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 a 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, consistent with an increase in gene transcription, confirmed by RNA-seq. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, high- est 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 promoters (84% Cd11b+Gr1+ in WT vs 4% in OE-HMGN1, p<0.002; Fig A).

Discussion: Gain of chromosome 21q22 is the most frequent molecular feature in complex karyotype AML. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, high- est 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 promoters (84% Cd11b+Gr1+ in WT vs 4% in OE-HMGN1, p<0.002; Fig A).

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.

Figure 1.

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 macrophage function in patients with thrombocytopenia (Towsley DM et al. ASH 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/reapsed ITP patients. Participants were at least 18 years of age, had a platelet count of less than 30×10^9/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: A multicentre prospective study was performed in non-splenectomized corticosteroid resistant/reapsed ITP patients. Participants were at least 18 years of age, had a platelet count of less than 30×10^9/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.

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 potential promising therapeutic option for patients with severe, corticosteroid-resistant or relapsed ITP.

S432
NOVEL PERSPECTIVES IN GENOTYPE-PHENOTYPE CORRELATIONS IN MYH9-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
Aims: To test the hypothesis that mutations in the regulatory domain of MYH9 gene are associated with severe thrombocytopenia and platelet dysfunction.

Methods: Whole exome sequencing was performed in 86 propositi with chronic thrombocytopenia. Results: WES detected a monoallelic loss-of-function mutation in the THPO-MPL axis in 3 propositi. Conclusion: Monoallelic mutations in the THPO-MPL axis are associated with severe thrombocytopenia and platelet dysfunction.

Aims: To investigate the role of GFI1B in megakaryocytes differentiation and function.

Methods: GFI1B expression was tested in patient iPSC derived megakaryocytes. Results: GFI1B mRNA expression was increased in cells derived from individuals with both C168F and CH294fs mutations. Conclusion: GFI1B is a critical regulator of megakaryocytes differentiation and function.

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Methods: Whole exome sequencing was performed in 86 propositi with chronic thrombocytopenia. Results: WES detected a monoallelic loss-of-function mutation in the THPO-MPL axis in 3 propositi. Conclusion: Monoallelic mutations in the THPO-MPL axis are associated with severe thrombocytopenia and platelet dysfunction.

Aims: To investigate the role of GFI1B in megakaryocytes differentiation and function.

Methods: GFI1B expression was tested in patient iPSC derived megakaryocytes. Results: GFI1B mRNA expression was increased in cells derived from individuals with both C168F and CH294fs mutations. Conclusion: GFI1B is a critical regulator of megakaryocytes differentiation and function.
Background: ITP is characterized by autoantibody-directed platelet destruction mediated by activated monocye 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 ITT 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 (ptt) counts (<30K/μL) were enrolled (76 in S047, 74 in S048) in a 2:1 randomization to FOSTA 100mg or placebo bid, and stratification by prior splenectomy and baseline plt ct<or ≥15K/μL. Sixty-one % of pts were female; median age was 54 y (20-88); 93% were Caucasian; and stratification by prior splenectomy and baseline plt ct<or ≥15K/μL. Sixty-one % of pts were female; median age was 54 y (20-88); 93% were Caucasian; 93% had cITP; median disease duration: 8.5 y; median baseline plt ct: 16K/μL.

Results: Across both studies, a SR occurred in 18/101 (18%) FOSTA vs 1/49 (2%) placebo pts, but not in the 29 responders. The number of pts with ≥1 adverse event (AE) was similar in FOSTA vs placebo (54% vs 52%). The majority AEs on FOSTA were mild or moderate; all resolved over time. Most common AEs were: diarrhea (29% vs 15%), nausea (19 vs 8%), hypertension (20 vs 8%), ALT/AST increase (10% vs 0%). Serious AEs were reported in 13% FOSTA vs 21% placebo pts.

Summary/Conclusions: Fostamatinib substantially improves plt tcts 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

Acute lymphoblastic leukemia - Biology

S436

THE YING AND YANG OF JAK SIGNALING : LOSS OF USP9X BUFFERS JAK SIGNALING AND ENHANCES SURVIVAL OF CRLF2-JAK-STAT EXPRESSING B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Children with Down syndrome (DS) are prone to development of high risk B cell precursor (BCP) acute lymphoblastic leukemias (DS-ALL) that differ genetically from most sporadic pediatric ALLs. Chromosomal rearrangements causing increased expression of CRLF2, the receptor for thymic stromal lymphopoietin (TSLP), characterize about half of DS-ALLs. Aims: Understanding the pathogenesis of relapse of DS-ALL relating to their CRLF2 status.

Methods: Integrative genomic analysis of matched diagnosis remission and relapse DS-ALLs, pharmacological inhibition and genetic CRISPR mediated silencing.

Results: Genomic analysis of 25 matched diagnosis remission and relapse DS-ALLs, pharmacological inhibition and genetic CRISPR mediated silencing.

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Results: Genomic analysis of 25 matched diagnosis remission and relapse DS-ALLs, pharmacological inhibition and genetic CRISPR mediated silencing.

These observations suggest that genetic or pharmacological restraining of JAK-STAT signaling may be beneficial to leukemic B cell precursors by enhancing the fitness of JAK-STAT driven CRLF2 mutated leukemic cells. Both pharmacological and genetic CRISPR mediated silencing of USP9X reduced STAT5 phosphorylation and enhanced the survival of CRLF2-JAK2R683Gs transduced ALL cells. To test directly the effect of JAK inhibition, we treated CRLF2/JAK2R683G transduced cells with increasing doses of ruxolitinib, an JAK inhibitor currently in clinical trials for CRLF2-JAK-STAT ALLs. Strikingly while high doses (>2μM) were cytotoxic, low doses (0.25μM) enhanced the survival of CRLF2-JAK2R683Gs expressing ALL cells. Summary/Conclusions: These observations suggest that genetic or pharmacological restraining of JAK-STAT signaling may be beneficial to leukemic B cell precursors by enhancing the fitness of JAK-STAT driven ALL. This and the reduction of JAK-mutated clones at relapse suggest that pharmacological effect of JAK2 specific inhibitors may be limited. Rather, combined silencing inhibitors or direct targeting of the TSLP receptor may be a useful therapeutic strategy for DS-ALL.
TNF 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 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 survival to cell death and characterizes a distinct vulnerability in ALL.

S438
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 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 genonomic 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 cMyc in the initiation and maintenance of T-ALL.

Methods: To evaluate if cMyc could act as a bona fide oncogene in the pathogenesis of T-ALL, we developed a conditional R26-driven cMyc overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the cMyc gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombine-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 VavCre mice, in order to obtain hematopoietic specific expression of Myb and the EGFP/luciferase from the ROSA26-promoter. Notably, Vav-iCre+ R26-Myb+/tg 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 cMyc and lack Pten in developing T-cells and found that cMyc 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 cMyc 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.
Background: Several somatic ribosomal 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 Ba/F3 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.

Figure 1. The differential proteome screen revealed overexpression of several Jak-Stat signaling components (Csf2rb, 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 mouse model. The relevance of this overexpression was illustrated by enhanced Jak-Stat pathway activation upon cytokine stimulation in RPL10 R98S lymphoid cells, as well as increased sensitivity of these cells to clinically used Jak-Stat inhibitors ruxolitinib and pimozide. RPL10 R98S positive leukemia patients likewise showed overexpression of IL7RA, Jak1 and STAT5, increased sensitivity to pimozide, as well as a mutually exclusive mutation pattern between RPL10 R98S and Jak-Stat lesions, suggesting that RPL10-R98S also modulates the cascade in human T-ALL. Programmed -1 ribosomal frameshifting (-1 PRF) recently emerged as a post-transcriptional mechanism regulating expression of cytokine receptors. We identified -1 PRF signals in mouse and humanJak-Stat genes and observed RPL10 R98S associated frameshifting reduction in several of these, which may contribute to their overexpression. Altered levels of -1 PRF can however only partially explain observed Jak-Stat protein expression changes, and transcriptional changes and altered protein stability are also involved. Indeed, our data point to altered protease activity and composition in RPL10 R98S cells, with upregulation of immunoproteasome specific catalytic subunits, which may explain the increased stability of particular proteins such as Jak1. Of further medical interest, RPL10 R98S cells showed reduced protease activity and enhanced sensitivity to the clinically used protease inhibitors bortezomib and carfilzomib.

Summary/Conclusions: We explored the molecular mechanism by which the RPL10 R98S mutation contributes to the pathogenesis of T-ALL. We propose a model in which R98S associated decreases in -1 PRF levels, combined with changes in the degradation of particular proteins and potential other mechanisms such as transcriptional regulation, leads to selective upregulation of the Jak-Stat cascade (Figure 1). Besides expanding the relevance of the Jak-Stat cascade in T-ALL and leukemia in general, our results have therapeutic potential since cells harboring the RPL10 R98S mutation are sensitized towards clinically used Jak-Stat and protease inhibitors.

NFATC3-PLA2G15 IS A NOVEL INTERGENICALLY SPliced ChimerA THAT IS ASSOCIATED WITH Agressive t-ACUTE LyMPHOBLASTIC Leukemia BIOlogy

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Background: Transcriptional read-through of a single mRNA between contiguous loci, or cis-spaying of adjacent genes (cis-SAGE), results in transcription of intergenically-spliced chimeric RNAs (ISCs) in the absence of structural genomic changes. Recent advances in high-throughput RNA-sequencing analysis have permitted identification of aberrant ISC expression as a potential cancer driver, but knowledge of leukemia-related ISCs is lacking.

Aims: To examine whether cis-SAGE generates biologically important ISCs in T-acute lymphoblastic leukemia (T-ALL).

Methods: We performed RNA-sequencing of 12 cases of T-ALL and normal thymic RNA, and used targeted analysis pipelines to detect T-ALL-specific fusion chimeras.

Results: We identified 140 T-ALL-specific fusions, of which 55 involved genes located within 30kb of each other, in the same transcriptional orientation. This distance is consistent with that previously observed for cis-SAGE, suggesting that ISC expression is common in T-ALL. In total, putative ISCs were detected in 10/12 samples, with a median of 4 (range 0-15) per patient. We performed further analysis on the candidate ISC NFATC3-PLA2G15, which includes the Nuclear Factor of Activated T-cells (NFAT) family member NFATC3, a critical regulator of normal thymopoiesis and known modulator of T-ALL biology. We found that primary T-ALLs exhibited a wide range of NFATC3-PLA2G15 expression, while levels in normal tissue were either very low or undetectable. 5’ RACE PCR analysis of leukemic cDNA revealed that fusion transcription was initiated in exon 1 of NFATC3. We also performed array competitive genomic hybridization of 115 diagnostic T-ALL samples, and found no evidence of microdeletions that would result in NFATC3-PLA2G15 expression, providing strong evidence that NFATC3-PLA2G15 is a true ISC that is generated by cis-SAGE. We found that the NFATC3-PLA2G15 fusion had lower activity than wild-type NFATC3 in both luciferase reporter experiments and proliferation and survival complementation assays in NFAT-null ALL cell lines in vitro. Gene set enrichment analysis revealed that primary T-ALL blasts with elevated NFATC3-PLA2G15 levels had reduced transcription of canonical NFAT target genes in vivo, suggesting that these cases may have lower activity of normal physiological NFAT pathways. Strikingly, we found that higher NFATC3-PLA2G15 levels strongly correlated with both shorter time to leukemia development (p=0.01) and survival (p=0.003) in patient-derived T-ALL xenografts in immunodeficient mice. These findings were corroborated by survival analyses of human T-ALL patients treated as part of the Francophone multinational GRAALL-2003 and -2005 studies, as cases with the highest quartile of NFATC3-PLA2G15 expression had significantly reduced 5 year overall survival (52.6%, 95% CI 33.3% - 68.7%) compared with NFATC3-PLA2G15 low cases (69.8%, 95% CI 58.8% - 78.3%, p=0.047).

Summary/Conclusions: Our results suggest that ISC expression is common in T-ALL, and that high expression of the NFATC3-PLA2G15 ISC correlates with reduced canonical NFAT pathway activity and poor patient outcome.
**Thrombotic disorders**

**ASSESSING THE RISK-BENEFIT OF ANTICOAGULANTS IN ELDERLY PATIENTS WITH CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: A POPULATION BASED STUDY**

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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 chemotheraphy agents with the associated risk for thrombocytopenia. However, the consequences of a recurrent VTE or a major bleeding (MB) event might be different in terms of mortality. As a result, the risk of VTE recurrence or a MB event may 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.

Aims: To estimate the case fatality rates of VTE recurrence and MB, as well as the case fatality rate-ratio for MB and VTE recurrence in cancer patients developing a VTE treated with anticoagulants.

Methods: We conducted a retrospective population-based cohort study in Ontario, Canada using de-identified linked administrative healthcare databases housed at the Institute for Clinical Evaluative Sciences (ICES). We included patients over 65 years of age with a diagnosis of cancer defined using provincial, ICD-9 and ICD-10 codes for major malignancies and who developed a VTE event within 6 months of the initial cancer diagnosis. VTE was identified through a Cox proportional hazards model using a combination of diagnostic codes for deep vein thrombosis (DVT) and pulmonary embolism (PE) and codes identifying diagnostic procedures for VTE (i.e. ultrasound, CT pulmonary angiography, lung scintigraphy) within 7 days of each other. Recurrent VTE and MB events were assessed within 180 days from the index date. MB was identified using a previously validated algorithm and included upper and lower gastrointestinal and intracranial bleeding events. Treatment was classified based on the first available prescription within 7 days of the index VTE. We estimated mortality within 7 days of the VTE recurrence or MB events using an unadjusted and Cox proportional hazards model and competing risk analysis. Ratios of the mortality for MB compared to VTE recurrence were calculated and 95% confidence intervals were estimated using non-parametric models.

Results: Between 2004 and 2014 there were 6967 VTE events identified in cancer patients over 65 years of age and treated with an anticoagulant. Mean age was 75 years, and 47.6% patients were women. Of all patients, 59.9% received prescriptions for LMWH alone, 15.3% for LMWH followed by warfarin, 22.1% for warfarin and 2.7% for rivaroxaban. At 180 days after the index VTE event there were 235 (3%) MB events and 1184 (17%) VTE recurrences. Within 7 days of the outcome event there were 26 (11%) deaths after MB and 6 (0.5%) after VTE. The mortality ratio for MB versus VTE was 21.8 (95% CI 9.53-53). In exploratory analyses we did not find differences according to type of anticoagulant prescription.

Summary/Conclusions: 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.

**COMPARATIVE ANALYSIS OF PREDICTIVE MODELS FOR THROMBOEMBOLIC EVENTS IN LYMPHOMA PATIENTS**

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Background: Lymphoma is a malignancy that originates in lymph nodes and lymphoid tissue. The main category of lymphomas is non-Hodgkin’s lymphoma (NHL). NHL comprise about 3% of all cancers in Sweden. Some are aggressive and fast growing, while others are more indolent and do not necessarily require treatment. It is well known that cancer increases the risk of thrombosis, especially in the case of thromboembolism, but data are scarce on the risk of thrombosis in NHL patients.

Aims: The aim of this study is to evaluate the risk of thrombosis in NHL patients compared to controls and to study time trends in the risk of thromboembolism with recent advances in the treatment of these diseases.

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, foetal, 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: 1.54; 95% CI: 1.51-1.59); arterial thrombosis (HR: 1.58; 95% CI: 1.53-1.62); and pulmonary embolism (HR: 1.53; 95% CI: 1.49-1.59). 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, independent of previous history of thrombosis (HR: 1.64; 95% CI: 1.59-1.69) no previous history (HR: 1.43; 95% CI: 1.37-1.50) for previous history of thrombosis). The incidence of thrombosis for NHL patients started to increase about five months before the diagnosis of NHL, and reached its peak one month after diagnosis. The incidence stayed increased for the first year post diagnosis.

Summary/Conclusions: 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.

**S445**

**IMPACT OF A NEW ELECTRONIC ALERT SYSTEM (V2.0) FOR VENOUS THROMBOEMBOLISM PREVENTION IN HOSPITALISED CANCER PATIENTS**

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**Background:** Onco-hematologic hospitalised patients constitute a group at high risk of venous 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 prophylaxis 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 were performed. ThroLy prediction tool was validated by univariate logistic regression analysis, while the model was developed from a derivation cohort, which included 1251 patients were included, 782 patients in the first period and 469 in the second one.

**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).

**S444**

**IDENTIFICATION OF A NEW AND RELATIVELY FREQUENT SERPINC1 GENE DEFECT CAUSING ANTITHRROMBIN 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...
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 second patient (P2) had a new 863 bp duplication in tandem of exon 6. Sanger sequencing of the specific amplicons in the two cases with tandem duplication of exon 6 revealed Alu sequences surrounding these duplications. Finally, one out of 5 cases with 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 downstream exon 6 makes this region a hotspot for unequal recombination and 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.

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 inflammation 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 pathway 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 exploited 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 pathophysiological mechanic studies on IECs in experimental mouse models (MHC-mismatched and minor histocompatibility antigen (mHIA)-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 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.
Aims: 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, in vivo activated T cells were clonally isolated and tested for reactivity against a panel of target cells, including patient and donor derived hematopoietic cells, third party hematopoietic cells as well as different GVHD target cells (patient skin fibroblasts, third party colon carcinoma cells, biliary epithelial cells and lung fibroblasts) expressing the mismatched, patient variant, HLA-DP molecule.
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*03:01 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 LYMPHOID CELLS
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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 CD4+CD25+ T 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 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 ICAM/VCAM expression, 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 ALLOGENIC 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 had impaired BM endosteal 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, Th2 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, Th2, Th2, Th17, Th17 cells and Tregs, between patients with PT and GGF after allo-HSCT.
Methods: This prospective nested case-control study enrolled 20 patients with allo-HSCT matched patients with PGF, 40 matched patients with GGF after allo-HSCT, and 20 healthy donors (HD). Th1, Th17, Th1, Th2, Th2 cells and regulatory T cells (Tregs), are involved in the pathogenesis of PGF remains to be explored.
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 Th17 cells (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 Tc2 (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 Th1 cell/Tc2 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. 1.6% 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.
Background: Aberrant B-cell homeostasis has been described in patients (pts) with chronic graft-versus-host disease (cGVHD) following allogeneic stem cell transplantation (allo-SCT). However, there is no information on the predictive value of specific B-cell subsets of the incidence 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 consenting 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+), switched (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 mycopheno- late mofetil for GVHD prophylaxis. Diagnosis was myeloid (61%) or lymphoid (34%) malignancy in the majority of pts. Grafts 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 had undergone allo-SCT. However, there is no information on the predictive value of specific B-cell subsets of the incidence of cGVHD. cGVHD diagnosis was based on the 2014 National Institutes of Health guidelines.

Figure 1.

Summary/Conclusions: In conclusion, D100 frequency of naïve and switched B cells predicts the subsequent development of cGVHD. Lymphoid malignancies and older age may be associated with aberrant B-cell reconstitution. Consideration of D100 B-cell subsets may improve risk stratification models for the development of cGVHD.
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 improve quality of life for affected patients. Hematopoietic allogeneic stem cell transplantation (HSCT) has the potential to cure the disease. However, there is little experience in applying HSCT in PKD and guidelines are not available. To date, only four European patients survived. Patients treated in Asian hospitals differed from European patients in that they were younger (p=0.001), less often splenectomized (p=0.048) and had a lower ferritin level before transplantation (p=0.048). They were more often transplanted with peripheral blood stem cells (p=0.014) and more often conditioned on a cyclophosphamide (p=0.007) regimen.

Summary/Conclusions: This is the first study on outcome of HSCT in PKD with enough cases to draw conclusions on the safety of HSCT in PKD can be drawn. However, we observed a better survival for patients transplanted before the age of ten. This difference could also explain difference in survival between patients transplanted in Europe versus Asia. The high rate of severe GVHD in this cohort is a reason for concern. The strong decline in survival of patients older than ten years of age indicates the need for very careful selection of HSCT-candidates.

Table 1.

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Results: From 1996 to 2016 a total of 16 PKD-patients were reported to have been treated by stem cell transplantation. Eight patients were treated in the EU and eight in Asian centres, respectively. No patient resulted to be transplanted in the US. Median age at transplantation was 6.5 years, (10 patients (62.5%) were <10 years; (37.5%) ≥10 years), seven patients (43.8%) were splenectomized at the time of HSCT. Fifteen patients (94%) reached engraftment. The sixteen patient showed mixed chimerism followed by spontaneous transition to full donor chimerism after splenectomy six months post transplantation. Two patients suffered from secondary graft loss. One of these had recovered 91% donor chimerism after donor lymphocyte infusion. Outcome in the other patient is unknown. GVHD grade 4 was reported in 6/16 cases (38%). There was no obvious relation between GVHD prophylaxis or any other clinical factors and the occurrence of GvHD grade 2-4 in our patients. Two-year cumulative survival was 74%. Two patients did not reach the two-year milestone yet. All five patients who did not survive died of transplant-related causes. Patients who did not survive were significantly older (p=0.036) and were all treated in a European center (p=0.026) (see Table). Also, they had suffered more often from GVHD grade 2-4 (p=0.031). Nine out of ten patients (90%) younger than ten years old survived splenectomy, whereas one out of five (20%) patients older than 10 years of age survived (p=0.036). Patients younger than ten years old were less often splenectomized (p=0.001), less often chelated (p=0.048) and had a lower ferritin level before transplantation (p=0.048). They were more often transplanted with peripheral blood stem cells (p=0.014) and more often conditioned on a cyclophosphamide (p=0.007) regimen.

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 xerocytosis (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 HX diagnosis in a retrospective diagnostic series of 103 patients and to determine the impact of free erythrocyte protoporphyrin (FEP). Methods: HX diagnosis was based on the typical left-shifted curve of osmolar gradient ektacytometry performed at CHU Biécoré from 1993 to 2016. All patients were from European origin. They were referred to our center for: chronic non-spherocytic hemolyisis (30), thrombotic 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 Deferprone (1). A perinatal edema history was noted in 17 (16.5%) patients. A history of thrombosis during the follow up in 12 (11.6%) 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 infaracts (2) and deep venous throm-
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Aims:
This paper reports results from the Phase II SUSTAIN study, which evaluated the use of crizanlizumab, a P-selectin inhibitor, in the treatment of sickle cell-related pain crises. The study was a randomized, double-blind, placebo-controlled trial in patients aged 16-65 years with sickle cell disease (SCD) who had experienced at least two sickle cell-related pain crises in the previous year.

Methods:
A total of 198 patients were included in the study, and they were randomized to receive crizanlizumab 5.0 mg/kg, 2.5 mg/kg, or placebo. The primary endpoint was the proportion of patients who were SCPC event-free at week 50. Additional endpoints included the proportion of patients who were SCPC event-free at week 52,

Results:
Among the 198 patients included in the study, 62.6% experienced 2-4 SCPC events and 37.4% experienced 5-10 SCPC events in the previous year. The proportion of patients who were SCPC event-free at week 50 was significantly higher in the crizanlizumab 5.0 mg/kg group (24/67; 35.8%) compared to the placebo group (16/65; 24.6%). In the crizanlizumab 5.0 mg/kg group, a greater proportion of patients were SCPC event-free compared to those in the placebo arm (28.0% vs. 4.2% and 31.9% vs. 17.0%, respectively).

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 also 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-αtreatment 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-αprimed 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).

Methods:
A randomized, double-blind, placebo-controlled trial in patients aged 16-65 years with sickle cell disease (SCD) who had experienced at least two sickle cell-related pain crises in the previous year was conducted. Patients were randomized to receive crizanlizumab 5.0 mg/kg, 2.5 mg/kg, or placebo. The primary endpoint was the proportion of patients who were SCPC event-free at week 50. Additional endpoints included the proportion of patients who were SCPC event-free at week 52, and the proportion of patients who were SCPC event-free at week 52.

Results:
Among the 198 patients included in the study, 62.6% experienced 2-4 SCPC events and 37.4% experienced 5-10 SCPC events in the previous year. The proportion of patients who were SCPC event-free at week 50 was significantly higher in the crizanlizumab 5.0 mg/kg group (24/67; 35.8%) compared to the placebo group (16/65; 24.6%). In the crizanlizumab 5.0 mg/kg group, a greater proportion of patients were SCPC event-free compared to those in the placebo arm (28.0% vs. 4.2% and 31.9% vs. 17.0%, respectively).

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 also 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.
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 cell nonpermeable 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 hemin (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 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 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/ unacceptable 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 very good partial response (VGPR) (ELd 112, Ld 89 [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
slightly higher incidence of infection with ELd (33% vs 26%). Overall rate (any grade of anemia, 84% vs 75%) and SPMd (11% vs 2%) was better for ELd vs Ld. However, exposure to ELd was longer than to Ld (median [Q1, Q3] treatment cycles: 19 [9, 42] vs 14 [6, 25]). Disease progression and infection were major causes of mortality in both arms; however, fewer deaths were reported with ELd vs Ld (165 vs 186).

Summary/Conclusions: At 4 years, ELd has the longest median follow-up of an immuno-oncology agent in MM. The data continue to show that adding elotuzumab 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 SPMd, was consistent with previous findings despite longer exposure, with minimal incremental AE compared with Ld therapy.

Study funding: BMS. Writing support: C Tomas, Caudex, funded by BMS.

S447
A PHASE IB STUDY OF ISATUXIMAB PLUS POMALIDOMIDE (POM) AND DEXAMETHASONE (DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) 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 (plus Pom/Dex) based on preliminary safety, efficacy, and PK data. Primary objective: 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 (2-11) 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 remained on treatment. 2 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 confusional state), and MTD has not been reached. Most common TEAEs, besides IARs, were fatigue (62%), diarrhea (35%), and dyspnea (31%). Most frequent Gr 3/4 hematologic abnormality (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 achieved at least PR (5, 8, and 3 pts at 5, 10, and 20mg/kg), including 1 CR, 8 PR, 1 CR and 7 PR. Clinical benefit rate (≥MR) was 73%. Median time to 1st response, 4.2 wks; median duration of response, 25.6wks. The PK parameters were comparable with those previously reported in the PFS interim analysis for ENDEAVOR.

Summary/Conclusions: ENDEAVOR was the first randomized phase 3 trial to directly compare two different PIs in RRMM. Patients who received Kd had significantly longer OS compared with patients who received Vd. Safety results were comparable with those previously reported. The combination of ISA and Pom/Dex showed promising clinical activity and manageable toxicity, with the potential for improved outcomes in RRMM.

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 Aims: To evaluate the efficacy and safety of carfilzomib (Kd) and pomalidomide (Pom) plus dexamethasone (VD) in relapsed or refractory multiple myeloma (RRMM) patients. Methods: The ENDEAVOR study was a phase 3, randomized, active-controlled study of DVd vs Vd. Patients who had RRMM and had received 1–3 prior lines of therapy were randomized in a 1:1 ratio to receive Kd or Vd. The Kd arm, carfilzomib was given on days 1, 2, 8, 9, 15, and 16 (20mg/m² on days 1, 2 of cycle 1; 9mg/m² thereafter) and Pom/dexamethasone (VD) on days 1, 2, 3, and 4. Results: 1,025 pts were randomized (479 Kd, 546 Vd). The primary endpoint was OS at 4 years. The OS benefit for Kd vs Vd was consistent in both high-risk and standard-risk MM, across the subgroups defined by cytogenetics (high-risk cytogenetics vs standard-risk cytogenetics), number of prior lines of therapy (1 vs ≥2 prior lines), and degree of prior chemotherapy (Table S1). The median OS (95% CI) was 47.6 (42.5-NE) months in the Kd arm and 40.0 (32.6-42.3) months in the Vd arm, and all-cause mortality was significantly reduced with Kd vs Vd (HR, 0.791; 95% CI, 0.648-0.964; 1-sided P=0.0100). The overall survival benefit was consistent regardless of prior bortezomib therapy (HR 0.75 for Kd vs Vd, no prior bortezomib; HR 0.84 for Kd vs Vd, prior bortezomib) and across all age groups (HR, 0.85 <56y; 0.71, 65-74y; 0.84, 75y+), baseline ECOG performance status (HR, 0.81, 0; 0.81, 1-2), and cytogenetic risk groups (HR, 0.83, high risk; 0.85, standard risk), and number of prior lines of therapy (HR, 0.83, 1 prior line; 0.76, 2-3 prior lines). The most frequent any-grade adverse events in the Kd arm were (Kd vs Vd) anemia (42.5% vs 28.3%), diarrhea (36.3% vs 40.6%), pyrexia (32.4% vs 15.4%), dyspnea (32.2% vs 13.6%), fatigue (32.2% vs 30.7%), and hypertension (32.2% vs 9.9%). Grade 3 or higher adverse events were experienced by 81.4% of patients in the Kd arm and 71.1% of patients in the Vd arm.

Summary/Conclusions: ENDEAVOR was the final randomized phase 3 trial to directly compare two different PIs in RRMM. Patients who received Kd had significantly longer OS compared with patients who received Vd. Safety results were comparable with those previously reported. The PFS interim analysis for ENDEAVOR is ongoing.

S459
EFFICACY AND SAFETY OF DARATUMUMAB, BORTEZOMIB AND DEXAMETHASONE (DVD) VERSUS BORTEZOMIB AND DEXAMETHASONE (VD) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED ANALYSIS OF CASTOR Aims: To evaluate the efficacy and safety of daratumumab (DAR) plus pomalidomide (Pom) and dexamethasone (Dex) in relapsed or refractory multiple myeloma (RRMM). Methods: The CASTOR study was a phase 3, randomized, active-controlled study of DVD vs VD. Patients with RRMM (any number of prior lines) were randomized in a 2:1 ratio to receive DVD or VD. The primary endpoint was OS at 4 years. The OS benefit for DVD vs VD was consistent in both high-risk and standard-risk MM, across the subgroups defined by cytogenetics (high-risk cytogenetics vs standard-risk cytogenetics), number of prior lines of therapy (1 vs ≥2 prior lines), and degree of prior chemotherapy (Table S1). The median OS (95% CI) was 47.6 (42.5-NE) months in the Kd arm and 40.0 (32.6-42.3) months in the Vd arm, and all-cause mortality was significantly reduced with Kd vs Vd (HR, 0.791; 95% CI, 0.648-0.964; 1-sided P=0.0100). The overall survival benefit was consistent regardless of prior bortezomib therapy (HR 0.75 for Kd vs Vd, no prior bortezomib; HR 0.84 for Kd vs Vd, prior bortezomib) and across all age groups (HR, 0.85 <56y; 0.71, 65-74y; 0.84, 75y+), baseline ECOG performance status (HR, 0.81, 0; 0.81, 1-2), and cytogenetic risk groups (HR, 0.83, high risk; 0.85, standard risk), and number of prior lines of therapy (HR, 0.83, 1 prior line; 0.76, 2-3 prior lines). The most frequent any-grade adverse events in the Kd arm were (Kd vs Vd) anemia (42.5% vs 28.3%), diarrhea (36.3% vs 40.6%), pyrexia (32.4% vs 15.4%), dyspnea (32.2% vs 13.6%), fatigue (32.2% vs 30.7%), and hypertension (32.2% vs 9.9%). Grade 3 or higher adverse events were experienced by 81.4% of patients in the Kd arm and 71.1% of patients in the Vd arm.

Summary/Conclusions: ENDEAVOR was the first randomized phase 3 trial to directly compare two different PIs in RRMM. Patients who received Kd had significantly longer OS compared with patients who received Vd. Safety results were comparable with those previously reported. The PFS interim analysis for ENDEAVOR is ongoing.

22nd Congress of the European Hematology Association

168 | haematologica | 2017; 102(2)
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^-4, 10^-5, and 10^-6) 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; 65% 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 (median: not reached vs 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^-4(P<0.0001), 10.4% versus 2.4% at 10^-5 (P<0.001), and 4.4% versus 0.8% at 10^-6 (P=0.01). 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.

Figure 1.

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.

S460

A PHASE 1B STUDY OF VENETOCLAX COMBINED WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA


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Background: Venetoclax (VEN) is a potent, selective, orally bioavailable small-molecular inhibitor of BCL-2. When combined, VEN can enhance the activity of bortezomib in multiple myeloma (MM) cell lines and xenograft models.

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: 0.3-29.8). 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 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.
Cyto genetic Complexity in Chronic Lymphocytic Leukemia: Definitions, Associations with Other Biomarkers and Clinical Impact; A Retrospective Study on Behalf of ERIC

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: Retrospective and prospective definitions for CK in CLL and systematic investigation of clinicobiological associations and prognostic impact.

Methods: 3850 CLL and monoclonal B-cell lymphocytosis (MBL) patients (CLL=3322, 93% and MBL=258, 7%, respectively) were analysed with Cpg-oligodeoxynucleotides/Interleukin-2 (CPG/iL2, n=379, 11%), phorbol-12-myristate-13-acetate (TPA, n=1345, 37%). CBA was mostly performed within the first year from diagnosis and before treatment administration (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 with no difference in the detection rate between different cell stimulation protocols. CK was significantly identified in 16% / del(11q): 377/3256 (12%)/ del(17p): 137/1345 (10%, respectively). The median OS (OS) is increased by 5-10% compared to those treated with chemotherapy alone. Patients with mutated IGHV genes (M-CLL) and/or unfavorable cytogenetic alterations (i.e. del(17p)/TP53 mutation, and del(11q)) have a better outcome than those with unmutated IGHV genes (U-CLL) and/or poor FISH cytogenetics and show a plateau in survival curves, suggesting that a fraction of these patients may have a survival similar to general population. Nevertheless, the possibility that some M-CLL patients without unfavorable cytogenetics are overtreated is of concern because of the treatment toxicity related to CIT, particularly FCR.

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 Institute, Milan, Italy for whom IGHV mutational status was available. Endpoints were OS and TFS.

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 cytogenetics 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 65% (CI, 52-79) for U-CLL cases (p<0.05). TFS at 5 and 10 years was 73% (CI, 69-77) and 68% (CI, 63-73) and 28% (CI, 33-37) and 10% (CI, 14-6) for M-CLL and U-CLL, respectively (p<0.05). After a median follow-up of 8 years (range, 1-26), 424 patients [161 M-CLL (163 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) required therapy. De novo treatment was initiated in 24% of cases (PA)-based therapy (n=83), alkylating agents (n=212), anti-CD20 mAbs with PA or bendamustine (n=75), anti-CD20 mAbs with alkylating agents (n=21), BCR-signal inhibitors or BCL2 antiapoptotic agents (n=9), others (n=23), and unknown (n=1).

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 65% (CI, 52-79) for U-CLL cases (p<0.05). TFS at 5 and 10 years was 73% (CI, 69-77) and 68% (CI, 63-73) and 28% (CI, 33-37) and 10% (CI, 14-6) for M-CLL and U-CLL, respectively (p<0.05). After a median follow-up of 8 years (range, 1-26), 424 patients [161 M-CLL (163 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) required therapy. De novo treatment was initiated in 24% of cases (PA)-based therapy (n=83), alkylating agents (n=212), anti-CD20 mAbs with PA or bendamustine (n=75), anti-CD20 mAbs with alkylating agents (n=21), BCR-signal inhibitors or BCL2 antiapoptotic agents (n=9), others (n=23), and unknown (n=1).
IBRUTINIB, FLUDARABINE, CYCLOPHOSPHAMIDE, AND OBINUTUZUMAB (iFCG) FOR PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) WITH MUTATED IGHV AND NON-DEL(17P)

Aims: To develop an FC-based chemoimmunotherapy regimen of finite duration that included ibrutinib 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 ibrutinib and a more potent antibody (obinutuzumab).

Methods: We designed an investigator-initiated phase II trial with ibrutinib, fludarabine, cyclophosphamide, and obinutuzumab (iFCG) for previously untreated CLL patients. The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through addition of ibrutinib and obinutuzumab. Key eligibility included age ≤ 18, IGHV-M, no del17p. Pts received 3 courses of iFCG. G-CSF was not mandated. Primary endpoint: CR/CRI with bone marrow (BM) MRD-neg (4-color flow-cytometry) after 3 courses of iFCG. Pts meeting primary endpoint received ibrutinib for 5 years and obinutuzumab for 3 years. A was added in cycle 2 with a dose ramp-up (to 400mg daily) over 5 weeks. A was added in cycle 2 with a dose ramp-up (to 400mg daily) over 5 weeks. A was added in cycle 2 with a dose ramp-up (to 400mg daily) over 5 weeks.

Results: Since activation (April 2016), 26 patients were consented; 23 initiated treatment. We report data on these 23 patients. This is the first report of this trial. Median age was 59 years (range, 25-71); there were 18 men. Prognostic markers included [FISH: del13q (n=17), negative (n=3); trisomy 12 (n=3); CD38+ (n=7); ZAP70+ (n=6/21 evaluated)]. By trial design, all patients had IGHV-M. Median B2M was 2.6 (range, 1.4-8.1). Median pretreatment WBC count was 81.1 K/uL (range, 3.1-224), platelet count 120 K/uL (range, 82-292), hemoglobin 12.4 g/dL (range, 9.1-15.6). Eighteen patients have completed 3 courses of iFCG and had initial response assessment (the remaining 5 patients have not yet completed 3 courses of treatment). All 18 patients achieved a clinical response; 14/18 (78%) achieved MRD-negative remission in the marrow at 3 months (17/18, 94%) and 16/18 (89%) achieved MRD-negative remission as their overall best response. Overall, 7/18 achieved CR/CRI with MRD-negative status in bone marrow at 3 months. All patients with PR had bulky adenopathy at baseline, and had residual lymphadenopathy ranging from 1.8 to 3.5 cm after 3 courses of iFCG. No patient has progressed, and all but one patient received treatment on protocol. Of the 23 pts, 11 pts had G3-4 neutropenia and 5 pts had thrombocytopenia. 1 pt had neutropenic fever. 1 pt who achieved MRD-cri CR developed pulmonary MAC infection, and declined further therapy. 1 pt had atrial fibrillation. G3 ALT developed in 3 pts. FC was dose reduced in 10 pts; ibrutinib dose-reduced in 2 pts.

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.

Table 1.

<table>
<thead>
<tr>
<th>ORR</th>
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<th>Marrow MRD</th>
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<td>7 (39)</td>
<td>7/11 (64) neg</td>
<td>9 (50)</td>
<td>7/9 (78) neg</td>
</tr>
</tbody>
</table>

Results: Between May 2015 and January 2016, 66 pts were enrolled. Two R/R pts died of a sepsis and 1 TN pt discontinued due to toxicity during the first induction cycle; these 3 pts with <2 induction cycles were excluded from the analysis as predefined by protocol. 34 pts were treatment-naive and 29 had R/R CLL (median number of prior therapies: 2; range: 1-8). Median age was 59 (28-77) years; the median CIRS score was 2 (0-14) and 16 pts (25%) had a creatinine clearance of 30-70/ml/min. 11 of 59 pts (19%) had a del(17p) and 45 of 61 (74%) had an unmutated IGHV status. Risk categories for TLS at baseline were: low (ALC ≤25,000/µL & LN <5cm): 9 pts (15%); intermediate (ALC >25,000/µL or LN 5-10cm): 35 (58%) and high (ALC >25,000/µL or LN >10cm): 16 (27%), 3 missing. 45 pts (71%) received B debulking, 18 (29%) pts immediately started with the induction. 60 pts completed 6 induction cycles with G and A. All TN (100%) and all but two of the R/R pts (93%) respond-
22nd Congress of the European Hematology Association
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 9th2017, 83 serious adverse events (SAEs)
were reported in 37 pts, including 69 SAEs (83%) related to study treatment.
66 SAEs (80%) were CTC°3-4 and 1 had a fatal outcome (sepsis in 4th induction cycle). 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 CTC°3-5) and hematological disorders (18 in 10 pts; 10
CTC°3-4), followed by infusion-related reactions (6 in 6 pts), laboratory TLS (5
in 5 pts; 1 during B debulking, 1 in induction cycle 1 with G, 2 in cycle 3 and 1
in cycle 4 with G and A) and ischemic coronary artery disorders (5 in 4 pts). No
clinical TLS occured.
Summary/Conclusions: With an ORR of 97% and a MRD negativity rate of
89% in pB at the end of induction phase this sequential treatment of B debulking, followed by G and A was very efficacious in a heterogeneous study population and well tolerated except for 3 fatal septicaemias in R/R pts.
S465

SAFETY RESULTS OF TERMINATED PHASE 2 STUDY OF IDELALISIB
PLUS RITUXIMAB IN TREATMENT NAÏVE CHRONIC LYMPHOCYTIC
LEUKEMIA (CLL) WITH DEL(17P)
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5Hospital of the University of Pennsylvania, Philadelphia, United States, 6University Hospital and Charles University, Faculty of Medicine, Hradec Králové,
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Background: Idelalisib (IDELA) is an oral PI3Kδ inhibitor approved in the EU
for use with rituximab (R) or ofatumumab in patients (pts) with previously treated
CLL or as first-line treatment of CLL with either del(17p) or TP53 mutation in
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.
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.
Methods: Treatment-naïve pts with CLL and confirmed del(17p) were treated
in a single arm study with R 375mg/m² IV weekly x8 and IDELA 150mg PO
BID continuously until disease progression or intolerability. Informed consent
was obtained. The study was fully enrolled when terminated early due to infection related safety concerns observed in a pooled analysis of ongoing Ph3
IDELA trials in front/early line therapy; the planned independent efficacy analysis was not performed, but investigator assessment is available.
Results: 102 pts (median age, 66; range, 37-86) were enrolled between Aug
2014 and Jan 2016 and received IDELA for a median (med) duration of 6.4
months (range, 0.7-17.0). The study was terminated in Mar 2016, >8 wks after
dosing of the last enrolled pt. 77 pts (75.5%) remained on study at the time of
study termination. The reasons for discontinuation from study were death
(4.9%), progressive disease (3.9%, 1 fatal), investigator discretion (9.8%), withdrawal of consent (2.9%), other anticancer therapy (2.0%), and lost to follow
up (1.0%). The investigator assessed response rate was 79%. 101 pts (99%)
had adverse events (AEs); Gr ≥3 occurred in 80.4%, the most frequent Gr ≥3
were ALT increased (27.5%), neutropenia (20.6%), infections (18.6%), and
diarrhea (14.7%). Laboratory Gr ≥3 ALT and/or AST elevations were seen in
41.2%, with med time of onset of 8.1 wks (range 4.1-24.1). The med age of pts
both with and without Gr ≥3 ALT/AST was 66 years, and the incidence of Gr
≥3 ALT/AST was similar in younger (43.9%, <65yr) and older (39.3%, ≥65yr)
pts. Gr ≥3 diarrhea/colitis occurred in 17.1% of pts <65yr and in 14.8% of pts
≥65yr. Dose interruptions due to AEs occurred in 71 pts (70%), most frequently
due to transaminase elevations (37.3%), and diarrhea/colitis (15.7%). Discontinuation due to AEs occurred in 27% of pts, most frequently due to ALT/AST
elevation (9.8%). Serious adverse events were reported in 46 (45.1%), including pyrexia (10.8%), diarrhea/colitis (11.8%). AEs of special interest included
Gr ≥3 infections in 20 pts (19.6%) of whom 5 had CMV and 3 had PJP (none
on prophylaxis), Gr ≥3 febrile neutropenia in 5 (4.9%) and any grade pneumonitis in 5 (4.9%). Of the 5 pts with CMV, all were CMV IgG+ at screening
and 2 also were IgM+. There were 6 on-study deaths, 3 associated with infection, 2 due to CLL progression and 1 due to heart failure.
Summary/Conclusions: In IDELA plus rituximab treated front-line CLL, the
pattern of AEs was similar to that seen in relapsed CLL studies at similar duration of therapy, however the frequency of Gr ≥3 ALT/AST was increased compared to the relapsed setting. There was no significant effect of age on the risk
of either ALT/AST elevations or diarrhea/colitis. The occurrence of CMV and
PJP infections is consistent with current IDELA labeling and speaks to the
potential benefit of risk mitigation through PJP prophylaxis and CMV monitoring
during treatment. NCT02044822.

172 | haematologica | 2017; 102(s2)

Aggressive Non-Hodgkin lymphoma Relapsed/refractory
S466

CLINICAL AND BIOLOGIC COVARIATES OF OUTCOMES IN ZUMA-1: A
PIVOTAL TRIAL OF AXICABTAGENE CILOLEUCEL (AXI-CEL; KTE-C19)
IN PATIENTS WITH REFRACTORY AGGRESSIVE NON-HODGKIN
LYMPHOMA (NHL)
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J. Timmerman11, P.M. Reagan12, A. Bot13, J. Rossi13, L. Navale13, Y. Jiang13,
J. Aycock13, M. Elias13, J. Wiezorek13, W.Y. Go13
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Background: Outcomes for pts with refractory aggressive NHL are poor with
current therapies (Crump, ASCO 2016). Results from the interim analysis of (n=62)
of ZUMA-1, the 1st multicenter trial of an anti-CD19 chimeric antigen receptor
(CAR) T cell, axi-cel, in refractory aggressive NHL, showed an objective response
rate (ORR) of 79% (complete response [CR] 52%; Blood 2016;128:LBA-6). Here
we present results from the primary analysis of ZUMA-1.
Aims: Here we present results from the primary analysis of the ZUMA-1 trial.
Methods: Pts received a target dose of 2 × 106 anti-CD19 CAR T cells/kg after
low- dose conditioning with cyclophosphamide and fludarabine. Eligible pts
(≥18 y) had diffuse large B cell lymphoma (DLBCL), primary mediastinal B cell
lymphoma (PMBCL) or transformed follicular lymphoma (TFL); an ECOG performance status (PS) 0-1; and refractory disease (progressive or stable disease
as best response to last prior therapy, or relapsed ≤12m of autologous stem
cell transplant [ASCT]). The primary endpoint for this analysis was ORR in the
combined DLBCL, PMBCL, and TFL population. Key secondary endpoints
were duration of response (DOR), overall survival (OS), and frequency of
adverse events (AEs). The primary analysis was triggered when 92 pts had at
least 6m of follow-up
Results: As of January 27, 2017, 111 pts from 22 institutions were enrolled;
101 pts (91%) received axi-cel. Median age was 58 y (range, 23-76), 67%
male, 85% stage III-IV, 47% IPI 3-4, 77% refractory to ≥2nd line of therapy,
and 21% relapsed ≤12m of ASCT. Axi-cel was successfully manufactured in
110/111 (99%) pts with an average turnaround time from apheresis to the clinical site of 17 d. With an ORR of 82% (n=92; P<.0001) the study met the primary
endpoint. The ORR in the mITT analysis set of 101 pts was 82% (CR 54%, PR
28%), and was consistent across key covariates including disease subtype,
refractory status, stage, and IPI score. At a median follow up of 8.7 m, 44% of
pts were in response and 39% were in CR. The median DOR was 8.2m overall
and not reached for pts who achieved a CR. Median OS was not reached;
80% of pts remained alive at 6 m. The most common Gr ≥3 treatment-emergent
AEs were neutropenia (66%), leukopenia (44%), anemia (43%), febrile neutropenia (31%), and encephalopathy (21%). Gr ≥3 cytokine release syndrome
(CRS) and neurologic events (NE) occurred in 13% and 28% of pts, respectively. All CRS and all NE resolved except 1 Gr 1 memory impairment. As previously reported, there were 3 Gr 5 AEs (3%). Peak CAR T levels and AUC
post–axi-cel were associated with durable responses. Additionally, this presentation will include an expanded analysis of efficacy outcomes by novel biologic and clinical covariates including key molecular phenotypes and tocilizumab/corticosteroid interventions used for management of adverse events.
Summary/Conclusions: Axi-cel significantly improved ORR in patients with
refractory aggressive NHL. The CR rate was 7-fold higher compared to historical controls (Crump, ASCO 2016) and nearly half the patients had an ongoing
response. Axi-cel demonstrated significant clinical benefit with a manageable
safety profile in pts lacking curative treatment options.
Drs Locke and Neelapu contributed equally to this study
S467

CC-122 IN COMBINATION WITH OBINUTUZUMAB (GA101): PHASE IB
STUDY IN RELAPSED OR REFRACTORY PATIENTS WITH DIFFUSE
LARGE B-CELL LYMPHOMA, FOLLICULAR LYMPHOMA, OR MARGINAL
ZONE LYMPHOMA
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A. Chiappella5, P. L. Zinzani7, R. Sarmiento8, S. Mosulen8, M. Petrarca9,
M. Pourdehnad9, K. Hege9, Z. Yang10, Z. Nikolova8, V. Ribrag1
1Institut Gustave Roussy, Villejuif, 2Institut Paoli-Calmettes, Marseille, France,
3Erasmus MC Cancer Institute, Rotterdam, 4On behalf of the LLPC (Lunenburg


S468

POLATUZUMAB VEDOTIN PLUS BENDAMUSTINE AND RITUXIMAB OR OBINUTUZUMAB IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA OR DIFFUSE LARGE B-CELL LYMPHOMA: UPDATED RESULTS OF A PHASE II STUDY

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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 were shown to have best response rates to date. The study is ongoing to establish the phase II recommended dose.

Table 1.

Table 1. Best Overall Response by PET/CT

<table>
<thead>
<tr>
<th></th>
<th>Pola+B (N=6)</th>
<th>Pola+B (N=6)</th>
<th>Pola+B (N=6)</th>
<th>Pola+B (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

S469

SINGLE ASENT 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. Maeroveot1, J. Westin2, C. Thielemont3, J. Zijlstra4, B.T. Hill5

Summary/Conclusions: Updated evaluation 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.
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 driven transcription, along with reductions in c-Myc and Bcl-2 family proteins.

Methods: PtS with R/R DLBCL were randomized to 60 or 100mg of SEL twice weekly (8 doses) per 28-day cycle. Pts were 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 dose groups (Grades 0-3) 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 (48% v 32%) were higher in the 100mg arm compared to the 60mg arm. Among the 63 evaluable pts (9 pts pending response), the ICRR determined ORR was 28.5% (Table 1). Nine responders, including 6 pts in CR, remained on treatment. Responders on the 60mg arm have a median time on treatment of 8.9 months as compared with 3.8 months on the 100mg arm.

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.

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 pts</td>
<td>63</td>
<td>19 (30.1%)</td>
<td>7 (11.1%)</td>
<td>11 (17.4%)</td>
<td>9 (14.2%)</td>
<td>27 (42.9%)</td>
</tr>
<tr>
<td>60 mg</td>
<td>32</td>
<td>10 (31.2%)</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>31</td>
<td>9 (29.0%)</td>
<td>2 (6.5%)</td>
<td>6 (19.3%)</td>
<td>6 (19.3%)</td>
<td>21 (67.7%)</td>
</tr>
<tr>
<td>GCB</td>
<td>24</td>
<td>13 (54.2%)</td>
<td>2 (8.3%)</td>
<td>5 (20.8%)</td>
<td>4 (16.7%)</td>
<td>18 (75.0%)</td>
</tr>
<tr>
<td>Non-GCB Subtype</td>
<td>39</td>
<td>6 (15.4%)</td>
<td>5 (12.8%)</td>
<td>5 (12.8%)</td>
<td>5 (12.8%)</td>
<td>26 (66.7%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The combination of MOR208 plus LEN is well tolerated and shows promising activity in patients with R/R DLBCL. Accrual and follow-up of patients is ongoing, as are cell of origin and other biomarker analyses.
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-hydroxylutarate (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 exposed to 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.

Aims: Evaluate the maximum tolerated dose (MTD), pharmacokinetic (PK) and pharmacodynamic (PD) profiles, safety, and clinical activity of enasidenib in pts with mIDH2 advanced myeloid malignancies.

Methods: This phase 1/2 study included pts aged ≥18 years (yrs) with mIDH2- defined AML, or with mIDH2 MDS with refractory anemia with excess blasts, and ECOG PS scores ≤2. Pts were relapsed or refractory (R/R) to prior anti-cancer therapy, or had untreated AML if aged ≥60 years and not eligible for standard-of-care treatment (Tx). Safety for all pts and clinical efficacy in the largest pt subgroup, those with R/R AML, from the phase 1 dose-escalation and expansion phases are reported.

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 1-4 adverse events (AEs) included thrombocytopenia (58%), neutropenia (31%), and anemia (27%). Thrombocytopenia was the most common reason for treatment interruption. Hematologic AEs were reversible, and grade >3 nonhematologic AEs occurred in ~10% of pts. Median time to first response was 1.9 months (mos); 87.3% of responding pts attained a complete remission [CR] by cycle 5. Median response duration was 5.8 mos. Of pts who achieved CR, 7 pts (21%) did so by cycle 3, 23 (68%) by cycle 5, and 28 (82%) by cycle 7. Median duration of CR was 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).

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 either dose-escalation doses combines well with time-matched decitabine (DEC) or azacitidine (AZA), 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-naïve pts ≥65 years old with AML. Eligibility included: ECOG PS ≤2; eligible for standard induction therapy; intermediate- or poor-risk karyotype. Pts received DEC (Arm D, 20mg/m²/day [d]; intravenous [IV]) on d 1-5, or AZA (Arm E, 75mg/m²/d; subcutaneous or IV) on d 1-7 of each 28-d cycle (C) in combination with once-daily oral 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: As of 13/09/16, 100 pts were enrolled in the expansion stage; 25 pts in each arm. Overall, 61% pts were male, 50% 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).

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 31%; secondary AML 44%; prior hypomethylating agent [HMA] 28%). AEs (all grade; severe; grade 3/4) included cytopenias (52%; 37%); neutropenia (26%); 17% grade 3/4). Among response-evaluable pts, those achieving an objective response had longer survival than pts who do not achieve an objective response (Figure 1).

Figure 1.

Summary/Conclusions: Overall, the safety profile was favorable when combining VEN at either dose with DEC or AZA in treatment-naïve 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.
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=12), 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); 1(0.1%) had 25% BM blast reduction, 5 pts (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, 1-4.5). The median OS among the CR/CRi/HI pts was 15.3 months (range, 2.27-17.17). HI pts was 5.7 months (range, 4.67-17.45), 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 AE 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 (P=0.10), CD8+ T-cells (P=0.02), and lower live CD4+Foxp3+PD1+ T-regulatory (T-reg) cells (P=0.01) inﬁltrate in BM. Patients who had a response had progressive increase in BM CD3+ cells and BM CD8+ cells, with increased ICOS (activation) marker on BM CD4-effecter 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.

Figure 1. OS with Aza+Nivo compared to historical survival with AZA-based salvage protocols in similar pts treated at MDACC in (a) all salvage and (b) ﬁrst relapse only.

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 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 therapies (consisting of 12 weeks of 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 (referring 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 56d, adjusted HR 0.38 (0.25-0.58) p<0.0001). A greater proportion of pts in AC220-002 proceeded to SCT: 23/58 (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 undoubtedly 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.
Immunotherapy in ALL

S476

GLOBAL REGISTRATION TRIAL OF EFFICACY AND SAFETY OF CTL019 IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/REFRACTORY (R/R) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): UPDATE TO THE INTERIM ANALYSIS

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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 were explored using summary statistics and graphical- and model-based analyses.

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=11]), 68 pts were infused with a single dose of CTL019 (median dose, 3.04 x 10^6/kg; range, 0.25-6.4 x 10^6/kg) and 29 pts were treated after manufacturing failures, 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 transplant (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–negative marrow. The release-free probability at 6 mo after remission onset was 75% (95% CI, 57%-87%; median DOR not reached). The probability of survival was 99% (95% CI, 77%-99%) 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 U Penn scale and managed using a protocol-specific algorithm; 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 or without other anti-cytokine therapy. Most common grade 3/4 hematologic AEs (>15%) other than CRS were hypotension (22%), hypoxia (18%), and infection (10%). Total cell count (N=78) included CD19+ lymphoblasts by morphology. CTL019 was manufactured from lymphodepleting chemotherapy in most pts (fludarabine/cyclophosphamide (AEs), and 4 pts (5%) were pending infusion at the time of data cutoff. Following

Results:

Summary/Conclusions: The ELIANA study confirmed the efficacy of a single infusion 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 LYMPHOBLASTIC 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 were explored using summary statistics and graphical- and model-based analyses.

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-
e
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 than 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 (r=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.

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<td>AUC0-28d</td>
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**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 or 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). Blinatumomab second and successive CTX salvage regimens (S2+) is poor compared with first salvage (S1) or frontline therapy, with less favorable outcomes among patients with shorter CR duration. 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 (3.3, 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 3 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.**

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**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 with >5% blasts as morphologic disease cohort. Response assessment occurred at 4 weeks. Median follow-up duration was 18 months (range, 0.2-57.3).

**Results:** 51 adults received 19-28z CAR T cells; 20 in the MRD and 31 in the therapy, with 95% confidence interval [CI]: 4.2-9.0) vs 6.3 months (95% CI, 4.8-9.0) (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 allelogeneic 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 haematologica | 2017; 102(s2) | 179
Biology and disease monitoring in CML

S481

A SECOND GENERATION LYSOSOMOTROPIC 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 lysosomal agonist 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 need to be investigated.

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-Tal-BCR-ABL). To accurately measure autophagy flow in long term LSCs in vivo, we generated the transgenic mouse Scl-Tal-BCR-ABL/GFP-LC3 by crossing the Scl-Tal-BCR-ABL model 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 CFSE®/CD34+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 (LTC-IC) 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-Tal-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+c-Kit+CD34+CD45+CFSEmax/CD34+CD133+cells respectively. 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-Tal-BCR-ABL). To accurately measure autophagy flow in long term LSCs in vivo, we generated the transgenic mouse Scl-Tal-BCR-ABL/GFP-LC3 by crossing the Scl-Tal-BCR-ABL model with a mouse bearing the autophagy marker GFP-LC3 fused to GFP.

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mal translocation (t(9;22)) that gives rise to the oncoprotein 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 imatinib effect on LSC. Subsequently, we identified the ITIM carrying Fc gamma receptor IIb (FcyRllb; CD32) to be a 2.8-fold upregulated in Bcr-Abl+ versus control LS K (lin; Sca-1+; c-kit+) cells using microarray and qRT-PCR.

Aims: In this study, we first aimed to validate Bcr-Abl mediated FcyRllb upregulation on mRNA and protein level in leukemic cells. Next, we tested the effect of shRNA mediated FcyRllb knock-down and depletion on cell line and patient derived CML cells for studying FcyRllb 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 SCLITa/Bcr-Abl BM using FcyRllb shRNA or scrambled control and subsequent transplantation, followed by analyses of the disease, including immune-pheno-typing, and protein expression as well as histological analysis.

Results: Bcr-Abl increased FcyRllb mRNA (13.2-fold, p<0.001) and protein expression in primary murine lineage negative (lin-) BM cells. Reduction of FcyRllb in immortalized SCLITa/Bcr-Abl progenitor cells significantly reduced CFC (colony forming unit) expression and CFU (colony forming unit) proliferation rate in these cells (2.27-fold, p<0.001). Moreover, transplantation of SCLITa/Bcr-Abl shRNA;FcyRllb 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 Gr-1+ cells (Gr-1+;CD45.1+;GFP+) were reduced in the BM (1.28-fold, p<0.01) 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;FcyRllb vs scrambled control. We observed similar effects upon FcyRllb depletion (FcyRllb-) vs wildtype (FcyRllb+/+) combined with virally induced Bcr-Abl expression. Interestingly, Bcr-Abl signaling induces FcyRllb phosphorylation in leukemic cells. Analysis of downstream signal pathways showed decreased levels of p-ERK, p-BTK, p-PLCγ1 in FcyRllb-/-, compared to FcyRllb+/+ Bcr-Abl transduced immortalized primary murine BM cells.

Summary/Conclusions: FcyRllb 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 FcyRllb is critical and disease specific making it a potential novel therapeutic target in CML stem cells.

S483 MYC-DEPENDENT REPRESSION MECHANISM OF THE MiR-150 TRANSCRIPTION REGULATION IN CHRONIC MYEOLOID LEUKEMIA (CML) P. Hronová1, C. Rytířová1, K. Kager2, K. Sovová1, F. Sovova1, H. Kamová1, H. Klámová2, P. Pecherková1, Z. Sovová1, J. Kobílčíková1, T. Stiplka2, D. Perrot3, K. Machová Poláková1,4
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Background: The expression of miRNAs is regulated at transcriptional as well as posttranscriptional levels. Dysregulation of miRNAs could directly induce or be a consequence of oncogenic pathways. Chronic myeloid leukemia (CML) is characterized by the 9;22 translocation resulting in the expression of Bcr-Abl tyrosine kinase which is the result of a block of miR-150 maturation and that miR-150 levels negatively correlated with MYC mRNA levels in CML HSPCs (p<0.001). Role of MYC in CML was further strengthened by imatinib induced downregulation and restored miR-150 levels in K562 and KCL22. Imatinib resistance in K562R and KCL-22R was characterized by further miR-150 downregulation. To assess the MYC role on regulating miR-150 levels we tested the MYC binding sites from the miR-150 locus after the imatinib treatment. We suggested potentially synergistic route for imatinib-induced BCR-ABL1 inhibition. This could be processed not only directly but also through an inhibition of a mutual positive regulatory loop between MYC and BCR-ABL1 (Xie et al. 2002). We also noticed dysregulation of miR-150 expression which is adjacent to the studied myri-150 CpG) to become activated by imatinib. We observed MYC levels dependent regulation of both genes, but FCGR1 is activated by MYC. This different regulatory role may be facilitated by the detected transcription factor CTCF binding to an insulator site between miR-150 promoter and the CpG. An activation of the insulator via CTFC binding could be an interaction between enhancers and promoters (Bell et al. 2000). CTFC was previously described to be an inhibitor of MYC transcription and we show CTFC transcription to be induced by imatinib. CTFC binding to DNA is prevented by DNA methylation. We did not detect DNA methylation within miR-150 upstream region.

Methods: We have used immortalized primary murine cells and HoxB8 immortalized murine bone marrow (BM) cells for studying FcyRllb 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 SCLITa/Bcr-Abl BM using FcyRllb shRNA or scrambled control and subsequent transplantation, followed by analyses of the disease, including immune-pheno-typing, and protein expression as well as histological analysis.

Results: Bcr-Abl increased FcyRllb mRNA (13.2-fold, p<0.001) and protein expression in primary murine lineage negative (lin-) BM cells. Reduction of FcyRllb in immortalized SCLITa/Bcr-Abl progenitor cells significantly reduced CFC (colony forming unit) expression and CFU (colony forming unit) proliferation rate in these cells (2.27-fold, p<0.001). Moreover, transplantation of SCLITa/Bcr-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 Gr-1+ cells (Gr-1+;CD45.1+;GFP+) were reduced in the BM (1.28-fold, p<0.01) 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 observed similar effects upon FcyRllb depletion (FcyRllb-) vs wildtype (FcyRllb+/+), combined with virally induced Bcr-Abl expression. Interestingly, Bcr-Abl signaling induces FcyRllb phosphorylation in leukemic cells. Analysis of downstream signal pathways showed decreased levels of p-ERK, p-BTK, p-PLCγ1 in FcyRllb-/-, compared to FcyRllb+/+ Bcr-Abl transduced immortalized primary murine BM cells.

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Support: LH15104 of MSMT and 00023736 of MZCR
and ii) verify accuracy and inter-laboratory reproducibility of results. The second phase of the study, involving 39 Italian Hematology Units, was meant to sharing a common protocol, a joint database for clinical and mutational data BCR-ABL1 KD mutation screening.

A next generation amplicon deep sequencing (Deep Seq) strategy for routine conducted to assess the feasibility, cost, turnaround times and clinical utility of kinase domain (KD) mutation screening is a precious tool for timely and rational (CML) patients (pts) on tyrosine kinase inhibitor (TKI) therapy, BCR-ABL1 Verona, Verona, 17Division of Hematology, Vicenza, 18Ospedale Dell’Angelo, Hematology/Oncology “L. e A. Seràgnoli”, Bologna, 2Department of Clinical... 23University of Bologna, Bologna, Italy

Background: Benchtop next generation sequencers are gradually replacing Sanger sequencers in diagnostics labs because of greater throughput, better sensitivity and increasing cost-effectiveness. In chronic myeloid leukemia (CML) patients (pts) on tyrosine kinase inhibitor (TKI) therapy, BCR-ABL1 kinase domain (KD) mutation screening is a precious tool for timely and rational therapeutic reassessment and is recommended in case of Failure and Warning. A multicenter, multilaboratory prospective study (NEXT-IN-CML) has been conducted to assess the feasibility, cost, turnaround times and clinical utility of a next generation amplicon deep sequencing (Deep Seq) strategy for routine BCR-ABL1 KD mutation screening.

Aims: The first phase of the study was aimed to i) create a network of 4 labs achieving MMR by 12 months, in comparison with 33% (20/60) of patients with e13a2 transcripts. DNA the expression of e13a2 BCR-ABL1 mRNA was lower than that of e14a2, an observation that requires confirmation. DNA methods were more sensitive: following the achievement of mRNA fell more rapidly than DNA, likely reflecting the time taken for normal haematopoietic cells to recover. At later time-points there was good agreement between methods, indicating that later reduction in BCR-ABL1 IS is closely related to depletion of leukemic cells. Normalised to BCR-ABL1 DNA the expression of e13a2 BCR-ABL1 mRNA was lower than that of e14a2, an observation that requires confirmation. DNA methods were more sensitive: following the achievement of UMRD by RQ-PCR patients could, on average, be monitored by DNA Q-PCR for an additional 5 months.

S485

ESTABLISHING A NATIONAL NETWORK OF LABORATORIES USING NEXT GENERATION AMPICON DEEP SEQUENCING FOR BCR-ABL1 KINASE DOMAIN MUTATION SCREENING: THE ‘NEXT-IN-CML’ STUDY

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Results: In the first phase, 504/512 amplicons were successfully generated and sequenced, with a median number of forward and reads of 1,757 range 544-5,838). In the 128 samples analyzed, 51/52 expected mutations were consistently detected by all 4 labs and quantitation of mutation load was highly reproducible across a wide range of frequencies (2%>100%). Three out of 4 labs failed to detect the 1% T315I+ dilution. In clinical samples, additional low burden mutations <3% were occasionally called by one or two labs only, suggesting that this value should be taken as a threshold below which mutation detection is not reproducible and sequencing artifacts and errors cannot be ruled out. In the second phase of the study, pts positive for mutations were 25/159 (16%; 23 Failures and 2 Warnings) by Sanger Seq and 52/159 (33%; 44 Failures and 8 Warnings) by Deep Seq. Among the pts with low burden mutations detectable by Deep Seq, 4 had a T315I+ 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 Intermediate Sokal risk group. The number of positive pts and the number of mutations per pt were not significantly higher in those receiving 2nd- or subsequent-line TKI therapy than in those receiving 1st-line TKI therapy. Compound mutations found were only found in 2 out of 52 mutated pts (both in blastic phase).

Summary/Conclusions: In the first 1-3 months BCR-ABL1 mRNA fell more rapidly than DNA, likely reflecting the time taken for normal haematopoietic cells to recover. At later time-points there was good agreement between methods, indicating that later reduction in BCR-ABL1 IS is closely related to depletion of leukemic cells. Normalised to BCR-ABL1 DNA the expression of e13a2 BCR-ABL1 mRNA was lower than that of e14a2, an observation that requires confirmation. DNA methods were more sensitive: following the achievement of UMRD by RQ-PCR patients could, on average, be monitored by DNA Q-PCR for an additional 5 months.

Summary/Conclusions: In the first phase of the study was aimed to i) create a network of 4 labs achieving MMR by 12 months, in comparison with 33% (20/60) of patients with e13a2 transcripts. DNA the expression of e13a2 BCR-ABL1 mRNA was lower than that of e14a2, an observation that requires confirmation. DNA methods were more sensitive: following the achievement of UMRD by RQ-PCR patients could, on average, be monitored by DNA Q-PCR for an additional 5 months.

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Prognostic markers and new treatment in MDS

S486

PATIENTS WITH IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE SHOW SIMILAR SURVIVAL PATTERNS AS LOW RISK MDS PATIENTS


Background: Cytopения 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 prognosis.

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=0.06).

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. Muta
tional screening seems promising in detecting patients at risk of progression, however, other biomarkers for prognostication are warranted.

S487

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 in MDS CD34+ cells after exposure and loss of response to HMA have been reported. Nivo and Ipi in mono- or combination therapy for HMA 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 #1: Nivo 3mg/kg iv Days 6 and 20; cohort #2: Ipi 3mg/kg iv on day 6; and cohort #3. 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 has 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). 35 of 63 pts had progressive disease at the end of cycle 3. The ORR was 8% (16/18) in the AZA+Nivo cohort, 6% (3/52) in the AZA+Nivo cohort, 6% (3/52) in the Nivo cohort, and 3% (3/93) in the Ipi cohort. 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), colitis (N=1), thyroiditis (N=2), pneumonitis (N=3), and nephritis (N=1). Early 8-week mortality occurred in 1 patient due to a 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 high-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.

S488

ORAL RIGOSERTIB COMBINED WITH AZACITIDINE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND MYELODYSPLASTIC SYNDROMES (MDS): EFFECTS IN TREATMENT NAÏVE AND RELAPSED/REFRACTORY PATIENTS

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Background: Outcomes of pts with AML 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 75mg/m2 iv daily days 1-5 of a 28 day cycle; cohort #1: Nivo 3mg/kg iv on days 6 and 20; cohort #2: Ipi 3mg/kg iv on day 6; and cohort #3: 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 has 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). 35 of 63 pts had progressive disease at the end of cycle 3. The ORR was 8% (16/18) in the AZA+Nivo cohort, 6% (3/52) in the AZA+Nivo cohort, 6% (3/52) in the Nivo cohort, and 3% (3/93) in the Ipi cohort. 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), colitis (N=1), thyroiditis (N=2), pneumonitis (N=3), and nephritis (N=1). Early 8-week mortality occurred in 1 patient due to a 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 high-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.
Background: Hypomethylating agents (HMA) such as azacitidine and decitabine remain the standard of care for the treatment of myelodysplastic syndromes (MDS) however, loss of response to therapy is associated with poor outcomes. Multiple studies have tried to identify biomarkers of response but the impact of the mutational architecture present at the time of diagnosis in response outcomes is unclear.

Aims: To evaluate the impact of the mutational architecture present at the time of diagnosis in response outcomes is unclear.

Methods: We evaluated 222 previously untreated patients with MDS or CMML that received HMA therapy at The University of Texas MD Anderson Cancer Center. Next generation sequencing analyzing a panel of 28 genes was performed prior to therapy with HMA. VAF estimates were used to evaluate clonal and subclonal relationships within each individual sample with clonal heterogeneity being defined in cases with Pearson goodness-of-fit p-values <0.05. Generalized linear models were used to study association of response rates (ORR=overall and CR=complete) and risk factors. Response was defined following 2006 IWG criteria.

Results: A total of 143 patients (79%) had MDS and 43 (19%) had CMML, including 108 (49%) with lower-risk based on IPSS and 114 (51%) with higher-risk disease. Therapy consisted in azacitidine monotherapy in 60 (27%) patients, decitabine monotherapy in 57 (26%), guadecitabine in 46 (21) and combinations in 59 (27%). The ORR was 61% (135/222) with 80 (36%) patients achieving CR. A total of 161 (73%) patients had at least one detectable mutation. Median number of mutations was 1 (range 0-5). Frequencies of detected mutations are shown in Figure 1A. 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%). Overall response was 75% for HMA-treatment-naïve pts and 62% in HMA-resistant pts. Correlative studies suggest that RIG has chromatin modifying effects in combination with AZA which may overcome clinical AZA resistance (Chaurasia EHA 2017). 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%).

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 naïve 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. This research will help reveal the pathogenesis of MDS from a new angle and provide new ideas for the diagnosis, treatment and prognosis evaluation of MDS.

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 RCMD patients, four RAEB-1 patients and four RAEB-2 patients, meanwhile, the miR223 expression status were tested in four kinds 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 was 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 (50μM,) was used to induce erythroid differentiation of K562 cells which carried miR223 overexpression We used flow cytometry method CD71 and CD235a makers and qRT-PCR(CD235 and r-globin) to detect the erythroid proliferation.

Results: 1. We verified miR-223 could target RPS14 by assay of luciferase activity. 2. miR223 expression was commonly mutated at diagnosis. 3. miR223 expression in MDS patients could provide a much deeper and broader understanding of clonal origin/hierarchy of relapse after allogeneic HCT. The present study aimed to evaluate mutation dynamics post-HCT using longitudinal NGS have not been thoroughly examined. We hypothesized that serial sequencing of pre-HCT and post-HCT in AML patients could provide a much deeper and broader understanding of clonal origin/hierarchy of relapse after allogeneic HCT. The present study aimed to evaluate mutation dynamics in AML using serial samples from pre- and post-HCT with respect to transplant outcomes, particularly overall survival (OS) and relapse.

Aims: To track origins of post-HCT relapse in AML using serial sequencing

Methods: 88 AML patients were enrolled and sequenced using an Illumina HiSeq 2000 sequencer (84 myeloid custom gene panel) on 419 bone marrow samples at diagnosis (n=88), pre-HCT (n=88), 21 days after HCT (n=88), and at relapse (n=20). Two patients relapsed by day 21. T-cell (n=80) and donor (n=57) were also sequenced. All computational and statistical analyses were performed using Python and R.

Results: The mean on-target coverage in 419 samples was 1773.7x. In total, we detected 217 mutations throughout the course of treatment in 79/88 patients (89.8%). NPM1 (26.1%), DNMT3A (26.1%), CEBPA (13.6%), IDH2 (13.6%), FLT3 (12.5%), and TP53 (11.4%) were commonly mutated at diagnosis. Unsurprisingly, most mutations appeared at initial diagnosis (200/217, 92.1%). Only 1, 2, and 14 mutations were acquired/selected at pre-HCT (0.5%), day 21 (9.9%), and relapse (6.5%) respectively. Most mutations were cleared at pre-HCT (mean mutation allele frequency (VAF) from 27.4% to 2.9%) and were further reduced after HCT (mean VAF from 2.9% to 0.7%) (Fig A). Leveraging

Figure 1.
significant decrease over time with ibr. Adverse events (AEs) were largely grade 1 or 2 events; AEs occurring in ≥20% of pts were fatigue, diarrhea, muscle
related to inflammation, fibrosis, and cGVHD. From all treated pts showed a
Significant activation of PLCγ1-Y783 in CD4 T-cells. Analysis of soluble plasma factors asso-
ciated with cGVHD involvement including skin, mouth, and gastrointestinal system showed
similar responses (~90%). Of 25 responders with ≥2 involved organs, 20 (80%) continued ibr.

Systemic conclusions: With an ORR of 67% and a sustained response rate of ≥20 weeks of 71%, treatment with ibr resulted in clinically meaningful and
durable responses in pts who failed at least 1 prior treatment for cGVHD. Most responders were able to reduce steroid dose. PD and biomarker changes support
a beneficial effect of ibr on immune cell subsets in pts with cGVHD. The
value of selecting pts who were treated with chemo before ibr was consistent with those previously reported for pts with B-cell malignancies and those seen in cGVHD pts on concomitant steroids.

Responsiveness in these pretreated, high-risk population support study of ibr for frontline treat-
ment of cGVHD.

S493
OUTCOMES OF NON T-CELL-DEPLETED HAPLOIDENTICAL HSCT VERSUS HSCT FROM MATCHED SIBLING DONORS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION, AN ALWP-EBMT STUDY

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is
the standard of care for patients (pts) with relapsed or refractory acute myeloid leukemia (AML). In pts lacking matched sibling (MUD), HSCT from haploidentical donors (HAPLO) is an emerging option

Aims: The aim of the study was to compare outcomes of non T-cell depleted HAPLO HSCT to those from MUD HSCT

Methods:registry included were adults with AML in first CR undergoing transplantation from HAPLO vs MUD from 2007-2015. Due to significant interaction between karyotype and donor type, int- and high-risk AML were studied separately. In addition because of some characteristic differences between the 2 groups the propensity score technique was used: 2 MUD were matched with each haplo. The following factors were included in the propensity score model: patient, year of HSCT, time from diagnosis to HSCT, conditioning (RIC), source of stem cells (BM/MP), cytogenetic group, patient and donor CMV serology status

Results: We identified 2654 pts (HAPLO=185; MUD=2469) for int-AML (HAP-
LO=122; MUD=1888) or high risk-AML (HAPLO=63; MUD=581). Median follow
up was 30 (1-116) months. Among 2654 pts, 74% received PCYT and 26% ATG. Conditioning regimen was myeloablative in 55% vs 52% (p=0.52) of HAPLO and MUD pts, respec-
tively. HAPLO pts had a longer interval from diagnosis to HSCT (6 vs 4 months; p<0.01), had more often high risk AML (34% vs 23%; p<0.01), bone marrow as stem cell source (49% vs 19%; p<0.01) and CMV positive donors (72% vs 61%; p<0.01). Graft failure occurred more frequently after HAPLO (3% vs 1%; p=0.002). For pts with int-AML CI of a gvhD and cGVHD was 29% vs 20% (p<0.03) and 30% vs 36% (p<0.02) in HAPLO and MUD pts, respectively. At 2 years, NRM and RI were 26% vs 10% (p<0.01) and 17% vs 20% (p=0.52). Incremental age was independently associated to lower LFS, OS, and non-hematological mortality. A longer interval from diagnosis to HSCT was asso-
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=0.26) in HAPLO and MSD pts; GRFS was 49% 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 grafts, while younger age and donor CMV status was associated with lower RI, higher LFS and OS. Results were confirmed in the analysis with the the propensity 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.

S494

INDIVIDUAL OUTCOME PREDICTION FOR MDS AND SECONDARY AML AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION BASED ON GENETIC, PATIENT- AND TRANSPLANTATION-ASSOCIATED RISK FACTORS

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Background: Prediction of individual outcomes after allogeneic hematopoietic cell transplantation (alloHCT) is difficult, as it is influenced by a multitude of risk factors.

Aims: To develop a tool that predicts individual outcomes of patients with myelodysplastic syndrome (MDS) or secondary acute myeloid leukemia from MDS (sAML) after alloHCT.

Methods: We integrated molecular data with available prognostic factors in patients undergoing alloHCT for MDS and sAML to evaluate their impact on prognosis. 304 patients with MDS or sAML who underwent alloHCT were accrued for mutations in 54 genes. We used a Cox multivariate model and competing risk analysis with internal and cross validation to identify factors prognostic of overall survival (OS), cumulative incidence of relapse (CIR) and non-relapse mortality (NRM).

Results: In multivariate analysis, mutated NRAS, U2AF1, IDH2, TP53 and/or a complex karyotype were significant prognostic markers for OS besides age above 60 years, remission status treated but not in CR, IPSS-R cytogenetic risk, HCT-CI >2 and female donor sex. Mutated NRAS, IDH1, EZH2 and TP53 and/or a complex karyotype were genetic aberrations with prognostic impact on CIR. No molecular markers were associated with the risk of NRM. The addition of molecular information significantly improved the risk prediction for OS and CIR as assessed by the Akaike information criterion. Internal and cross validation confirmed the robustness of our comprehensive risk model. We developed an interactive risk prediction tool to provide personalized predictions for OS, CIR and NRM outcome after alloHCT. An individualized prediction for a 53-year-old male with sAML with trisomy 11, mutated NRAS, IDH2 and DMMT3A and complete remission after double induction is shown in Figure 1. The probability of CIR at 2 years was 45% and the patient relapsed after 0.61 years. The probability of OS at 2 years was 41% and the patient died after 0.88 years.

Summary/Conclusions: We combine molecular, cytogenetic, patient- and transplantation associated risk factors into a comprehensive risk score to provide personalized predictions for outcome after alloHCT. Upon validation in larger patient cohorts, this will improve patient information before alloHCT and provide a platform to improve treatment strategies for patients with high risk of CIR or NRM.

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 iCas9 suicide gene) after αβ T-cell depleted haplo HSCT to facilitate immune reconstitution and GvL effect. BPX-501 T-cells are genetically modified with the Cas9 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: 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/I 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.
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 genetic gains remain to be identified.

Aims: To identify individuals with inherited susceptibilities to hematologic malignancies, the Hereditary Hematologic Malignancy Clinic (HHMCC) was established in April 2014 at The University of Texas M. D. Anderson Cancer Center. The clinic’s goal is to develop a research testing framework for patients with hematologic malignancies suspected to have inherited predisposition syndromes.

Methods: Individuals were referred to the HHMCC 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: Clinical genetic testing was performed in 572/152 individuals (37%), particularly in patients naive 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 TP53 mutations. Two adults (2%) were diagnosed with Diamond-Blackfan anemia and one child with Diamond-Blackfan anemia and 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 (Nobilet syndrome) 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 identifying 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 IMPLEMENTATION IN THE SCNIR EUROPE
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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 and molecular genetic subtype: ELANE, HAX1, G6PT, G6PC3, WAS, SBDS, TA21 and p14 or no identified mutation, respectively. Our aim is to assess the risk of leukemic transformation within these genetic subgroups.

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 TA21 mutations and 27 other rare gene mutations (e.g. p14, 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 leukemic transformation. The time between first detection of CSF3R mutations and onset 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>
<thead>
<tr>
<th>Gene Mutation</th>
<th>Patients</th>
<th>MDS/Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CN</td>
<td>449</td>
<td>49 (11.0)</td>
</tr>
<tr>
<td>ELANE</td>
<td>118</td>
<td>17 (14.3)</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>SLCA4</td>
<td>28</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>WAS</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>JAGN1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>gene mutations without leukemia*</td>
<td>36</td>
<td>16 (44.4)</td>
</tr>
<tr>
<td>unclassified</td>
<td>43</td>
<td>16 (37.2)</td>
</tr>
<tr>
<td>Total CN</td>
<td>91</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>ELANE CN</td>
<td>48</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>unclassified</td>
<td>43</td>
<td>16 (37.2)</td>
</tr>
</tbody>
</table>

*Gene mutations without leukemia: G6PC3 n=9, TA21 n=5, p14 n=4, digenic mutations n=4, CDH1 n=4, DKC4 n=3, germline 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 ECUCLIZUMAB 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 (NCT01374360) 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 (HD A).

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/nervi ecu-treated; no-HDA/ecu-treated; no-HDA/nervi 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, dysnea, 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 least follow-up visits or in patients with lack of 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.

Evaluating the effect of ecu in patients with PNH with or without high disease activity (HD A).
Results: 4717 patients were enrolled; of these, 2670 had non-missing data on euc and HDA status, and were included in the current analysis (HDA/euc-treated, n=785; HDA/never euc-treated, n=636; no-HDA/euc-treated, n=1111; no-HDA/never euc-treated, n=1138). Median (min, max) duration of follow-up after baseline was longer for the ecu-treated patients compared with the never ecu-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 ecu-treated cohort had high 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 ecu-treated patients (13.7%)). A similar disparity at baseline was also observed in the no-HDA population (33.0% vs 11.0%, respectively). Following ecu treatment, the divergence in the proportion of patients with MAVE has substantially narrowed for the HDA patients (3.9% for ecu-treated vs 3.3% for never ecu-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 HDA status, 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/ecu-treated group experienced a greater mean (SD) score improvement than the HDA/never ecu-treated group (4.1 [10.3] vs 0.5 [6.8] points).

Table 1.

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-lye thrombopoiesis and hematopoietic stem cells maintenance. Mutations in MPL can drastically impair its function and be a contributing factor in multiple hematologic malignancies, including congenital amegakaryocytic thrombocytopenia (CANT). CANT is a rare inherited syndrome characterized by thrombocytopenia at birth, progressing to bone marrow failure and pancytopenia. The functional impact of CANT mutations on Mpl is yet to be determined. Here we report unique familial cases of CANT presenting with a previously unreported MPL mutation: T814C (W272R) in the background of the activating MPL G117T (K39N or Baltimore) mutation.

Aims: To develop characterization of this novel MPL mutant and the use of genome editing as a novel therapeutic option for CANT. Methods: Human megakaryoblastic UT-7 and murine Ba/F3 cells stably expressing human wild-type (WT) Mpl or mutant Mpl fused to mNeonGreen were used as models. Confocal microscopy, proliferation and surface biotinylation assays, as well as co-immunoprecipitation and western blotting analysis, were used to elucidate the function and trafficking of Mpl mutants. Multiplex, flow-based, CRISPR-Cas9 gene editing was used to repair mutant MPL and rescue its function. Cord blood from the younger male sibling was used as a source of primary homzygous Mpl K39N/W272R CD34+ cells. CD34+ cells were edited using ribonucleoproteins electroporation followed by sequencing and functional assays such as flow cytometry and single colony assays.

Results: Consanguineous parents and their eldest daughter, all heterozygous for Mpl K39N/W272R, do not present any signs of disease. Their monzygotic twin daughters presented at birth with severe thrombocytopenia leading to a diagnosis of CANT type 1. Whole blood sequencing revealed a homozygous double Mpl K39N/W272R mutation, as their younger male sibling. One of the twins died after bone marrow transplant. Confocal microscopy shows that a significant fraction of chimeric WT Mpl protein reaches the cell surface. Significant surface expression is also noted for Mpl K39N. In contrast, the chimeric Mpl protein bearing the W272R mutation, alone or together with the K39N mutation, showed no detectable surface expression of the Tpo receptor while being strongly co-localized with ER marker calreticulin. Both WT and K39N-mutated Mpl were found signaling competent, while single or double mutants bearing W272R were unresponsive to Tpo. Tpo-induced signaling was partially rescued by overexpression of GRASP55 (forcing 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 response to Tpo, as assessed by flow cytometry. CRISPR-Cas9 edited 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 CANT. Function of the deficient Mpl receptor could be recovered using two complementary approaches: CRISPR-Cas9 genome engineering 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 tPSA<100 Å2. A high-resolution co-crystal structure of complement C5 shows a unique binding site on the 188 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 R885H/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.

Conclusion: This series of molecules has been profiled by SPR and Wieslab ELISA and exhibits oral bioavailability (%F~30-50) in pre-clinical species. The development of complement C5 inhibitor for the treatment of complement mediated disorders is an exciting new therapeutic modality to treat both rare and common conditions where there is a need for a novel therapeutic approach.
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-TRANSPLANT 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 followed 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:** A two-visit HRQoL instruments (EORTC QLQ-C30 and MY20) were administered 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, fatigue, pain, constipation, diarrhea, nausea/vomiting, insomnia, disease symptoms, side effects of treatment, and neurotoxicity were assessed for the following subscales; global QoL increased after 9ID to 12MT (MID range 7-13), pain decreased at every time point (MID range -21 to -23), disease symptoms decreased 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). **Difference between MPT-T and MPR-R:** In the MPT-T arm significantly (p<0.05) and/or clinically (mean score difference (MSD) ≥5 points) less pain and disease symptoms at 3ID, less fatigue at 3ID and 9ID, less diarrhea and less insomnia at all time points were observed. In contrast, patients on MPR-R reported better global QoL, better physical functioning and less pain at 12MT, in general less side effects of treatment, and less constipation and neuropathy separately, at all time points than patients treated with MPT-T.

**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 OBITUNUZUMAB-BASED AND RITUXIMAB-BASED THERAPY IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED INDOLENT NON-HODGKIN LYMPHOMA


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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 ≥7 cm), ECOG performance status 0–2, and requiring treatment according to GELF criteria. Pts were randomized 1:1 to R 375 mg/m² on day (D) 1 of each cycle (C) or G 1000 mg on D1, 5, and 15 of C1 and D1 of C2–8, for 6 or 8 cycles depending on chemo (CHOP, CVP 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 well-being, 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 arms experienced clinically meaningful improvements from baseline in LYMS scores (Figure), and the summary scales that included this subscale (TOI, Lym-Total). On each summary scale, ≥50% of patients in each arm reported clinically meaningful improvements. There were no clear differences between arms in HRQoL scores over the course of therapy.

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 further support the benefit of G-chemo over R-chemo in GALLIUM.

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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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 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 anttican therapy, 218 (78%) were receiving treatment at the time of survey and 54% had ongoing symptoms related to their treatment or cancer. 197 (71.4%) patients did not want 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, 2.9% were not aware and 1.8% did not respond. The cohort of patients who did or did not want access to a support group was analysed further. 88% of patients had been given a key worker (either a specialist nurse, 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% 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 2006 (n = 229) and not stated (n = 14). There was no difference in the three groups when asked if they wanted access to 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 effect 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 haematological 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.
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. Double lumen PICCs (5 Fr) were inserted in 70 patients, single lumen PICCs (4 Fr) were inserted in 5 patients, and triple lumen PICC (6 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 (untunneled heparin-coated Vialon 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 occurred in 4 patients (4 coagulase-negative staphylococci; 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 staphylococci, 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 x2 test; catheter-related bloodstream infections: 5% in the arm A vs. 19% in the arm B, p=0.007 by x2 test) (Figure 1). Questionnaire covering activities of daily living confirmed improvement of quality of life.

19% in the arm B, = 0.01 by χ2 test; catheter-related bloodstream infections: 5% in the arm A vs. 26% in the arm B, p=0.01 by x2 test; rate and improvement of quality of life for patients with acute leukemia.

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.

SS05
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 via Patient Health Questionnaire (PHQ-9), fatigue via Brief Fatigue Inventory (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, 889 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 (p=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=0.01), 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, <0.001), massage (p=0.01, 0.02), and strength training (p<0.003, 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.

Table 1.

<table>
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<th>Symptom</th>
<th>MPN-SAF TSS</th>
<th>QOL</th>
<th>PHQ-9</th>
<th>BFI-Usual</th>
<th>ET</th>
<th>PV</th>
<th>MF</th>
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<tr>
<td>Strength training</td>
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<td>Antidepressants</td>
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<td>0.63</td>
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<td>Meditation</td>
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<td>0.02</td>
<td>0.16</td>
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<tr>
<td>Breathing exercises</td>
<td>&lt;0.001</td>
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<td>0.12</td>
<td>0.87</td>
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<td>Overall quality of life</td>
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<td>0.15</td>
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<td>Support groups</td>
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<td>0.15</td>
<td>0.03</td>
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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.
Acute lymphoblastic leukemia - Biology 2

P506

T CELL EXHAUSTION CHARACTERIZED BY COMPROMISED MHC CLASS I AND II RESTRICTED CYTOTOXIC ACTIVITY ASSOCIATES WITH ACUTE B LYMWHOPLASTIC LEUKAEMIA 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 transplant- tation (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 hematolog- ical 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. Patients were grouped by the Institute of Hematol- ogy, Transplant. Protocol and post-transplant time were matched in relapsed and non-relapsed patients. Post-transplant time were matched as follows: ±14days within 12 months ±1months from 12 to18months, ±3months from 18 to 36 months, ±12months 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 Uni- versity People’s Hospital in accordance with the Declaration of Helsinki, phe- notypic 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 relapse set- tings. Moreover, both CD4+ and CD8+ T cells exhibited compromised prolifer- ative capacity, cytokine production and cytotoxic potentials such as degranu- lation and granzyme B production (preferentially on CD4+T cells) in relapsed patients. In addition, T cells in the tumor were 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-induc- tion therapy.

Summary/Conclusions: In conclusion, our study suggested that T cells expe- rienced exhaustion comprehensively functional impairment in B-ALL relapse settings after allo-HSCT and reversal of T cell exhaustion was asso- ciated 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-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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Background: B cell acute lymphoblastic leukemia (B-ALL) even in the modern era of tyrosine kinase inhibitors (TKIs). Relapse of Ph+ALL may result from the persistence of leukemia-propagating cells (LPCs), which are defined by their ability to initiate human leukemia and self- renew in immunocompromised mice. Using an anti-CD122-conditioned NOG/SCID xenograft mouse assay, LPCs were enriched and CD4+CD38-CD58- fraction in human Ph+ALL (Y.K., ..., X.J.H., Leukemia, 2014). Furthermore, a cohort study demonstrated that Ph+ALL patients with LPCs phenotype at diagnosis exhibited a significantly higher cumulative incidence of relapse than did the group with other phenotypes, even when receiving uniform front-line imatinib-based therapy pre- and post-allo-transplant (Y.K., ..., X.J.H., BMT, 2015). Therefore, it is imperative to identify novel therapeutic strategies based on LPCs to improve the prognosis of Ph+ALL patients.

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 gene expression profiles of 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 NOG/SCID xenograft mouse assay. Moreover, western blot analyses were performed on the BM cells harvested from the different groups of recipient mice.

Methods: 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 treatment with either nilotinib or ruxolitinib was achieved when the combination treatment was derived by the engulfment analysis of BCR/ABL 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 lower expression of phospho-CrkL, JAK2 and STAT5 activities at the molecu- lar 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.

P508

PREDICTING ANTI-LEUKAEMIA ACTIVITY OF THE B-2-SELECTIVE INHIBITOR ABT-199 IN BCP-ALL BY FUNCTIONAL ASSESSMENT OF APOPTOSIS SIGNALING

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Background: Although survival rates of pediatric BCP-ALL patients have con- tinuously improved during the past decades, therapy-related toxicity and relapse occurring in 10-20 % of patients are associated with poor outcome, clearly emphasizing the need of novel, targeted treatment strategies. Deregulated sur- vival pathways and cell death resistance contribute to treatment failure and reoccurrence of the disease. ABT-199 (venetoclax) is a small molecule inhibitor of BCL-2 demonstrating anti-cancer activity among different malignancies. How- ever, predictive biomarkers are required for up-front identification of patients who would benefit from BCL-2 directed therapies.

Aims: The aims of this study were to assess the efficacy of ABT-199 in BCP- ALL, to functionally evaluate factors mediating ABT-199 susceptibility or resist- ance and to identify markers indicative of favorable anti-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 apo- tosis 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 rpdx.

Results: Different sensitivities of ABT-199 were observed in a series of BCP- ALL pdx 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 region (EC50 <1μM) with four out of six cell lines and 20 of 27 pdx, while ABT-199 insensitivities with EC50s of more than 1μM were identified in 26% of pdx leukemias. ABT-199 induces apoptosis based on its ability to form a sub-micron BCL-2/BAX complex, sequestering pro-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 (t2 = 0.71, p = 0.006), highlighting the importance of
comprehensive assessment of the direct target molecule and additional resistance
mediating molecules. In line, MCL1 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 mem-
ers and BCL-2 gene expression profiling. Mitochondrial dependence on BCL-2 (mitochondrial prim-
ing 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 addiction to other BCL-2 family members,
including BCL-XL or MCL1. Finally, we evaluated prediction of in vivo ABT-199 sensitivity in a pre-clinical ALL pxd mouse model by func-
tional BH3 profiling. Strikingly, high mitochondrial BCL-2-dependency was clearly
associated with prolonged leukemia-free survival upon ABT-199-therapy (two pxd, 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 sensitivi-
ties characterized by the level of the target molecule but also other interacting
regulators. Functionally, mitochondrial BCL-2-dependence 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 pxd leukemias is predictable by mitochondrial BCL-2-dependence, emphasizing the utility of iden-
tification of patients and guidance of future clinical application by functional assessment of apoptosis signaling.

P509

CD45RA- MEMORY T CELLS EXPRESSING AN NKGD2-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 ther-
apieties are still unable to solve. One of the main NK cell activating receptors is
NKG2D. The role of NKG2D in leukemia cells was analyzed by flow cytometry
and could be

Aims: The aim of this study was to analyze the NKG2DL expression on pediatric acute leukemia cells and determine their susceptibility to an NKGD2 CAR
cell based immunotherapy.

Methods: The expression of NKGD2L 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 monoc-
clonal antibodies directed against MICA, MICB, ULBP-1, ULBP-2, 4-1BB, CD3-z.

Summary/Conclusions: The expression of NKGD2L was significantly higher in
healthy donors compared with leukemia patients. The highest NKG2DL expres-
sion was observed in NKG2D CAR expressing T cells.

P510

A BILINEAL ACUTE LYMPHOBlastic LEUKEMIA ORIGINATING AT A
COMMON LYMPHODENDRITIC PROGENITOR
A. Gonzalez-Munillo1,*, C. Sánchez-Valdepeñas1, C. Robledo2, A. Castillo3, J. Abad-Cabezas4, C. Hernandez-Marcues1, D. Ruano1, L. Madero1, J. Alonso2, M. Ramirez1
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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
bilineal T- and B-ALL

Methods: Bone marrow cells obtained at diagnosis were used for all studies. Immunophenotyping was done by flow cytometry. T- and B-leukemic cell
purification was performed by immunomagnetics methods and DNA extracted after-
ward. 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, Qiajen). Mutations were validated by Sanger sequencing. Myeloid and ery-
thyroid clonogenic progenitors were isolated from myeloidcellular 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 bone
marrow disease in an X-ray image and a mediastinal mass in the chest X-ray image.
Moreover, T-cell receptor (TCR) rearrangement was detected in purified (>95% true) 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.849G>T (p.K28N)
mutation in the H3F3A gene was detected in both the B-ALL and T-ALL sub-
populations, 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 multistep myeloid-lymphoid rather than a lymphoid-restricted progenitor as the cell origin of the leukemia. Therefore, we cultured myeloid/erythroid-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 bilineal 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 (CSPG2) 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. CSPG2 is
upregulated in B-ALL compared with normal B-cells, suggesting a critical role in
leukemia cell proliferation and metastasis. However, its clinical significance and
biological function is unclear.

Methods: CSPG2 transcript levels were measured by qPCR in 122 samples of B-ALL patients. The samples were classified into two groups according to
whether they achieved complete remission (CR) or not. DNA extracted, and assessed for the presence of the H3F3A p.K28N mutation by
Sanger sequencing.

Summary/Conclusions: Our results show that CSPG2 transcript levels relate
with adult B-cell ALL survival in vitro and could be a promising and safe immunotherapeutic approach for pediatric acute leukemia patients.
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% [86-96%]; P=0.007). In multivariate analyses a high CSRP2 transcript level was independently-associated with CIR (HR=5.32 [1.64-17.28]; 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

THEРАЕUTIC TARGETING OF PRE-B CELL RECEPTOR SIGNALLING IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

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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 otherwise-relapsed ALL.

Methods: ALL cell lines (Reh, Nalm-6, PreB 697 and its glucocorticoid resistant descendant, R3F9) and 36 primary-derived xenograft (PDX) cells from 16 ALL were used in the study. Cell viability was assessed by Resazurin. Pre-BCR expression (µHc, Vpreb and λ5) 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-Ig antibody, as well as drug pharmacodynamic measures (p-BTK, p-SYK, p- AKT, p-Erk, p-PLC-Y2, p-BLNK). Apoptosis and cell cycle were analysed by flow cytometry using Annexin V and Propidium Iodide. RQ-PCR was used to measure regulatory CSPR2 expression as a way to reverse drug resistance.

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 µM-5.83 µM). However, cells were resistant to Ibrutinib (mean GI50 15.9 µM, range 11.47 µM-18.3 µM) and CAL-101 (mean GI50 52.08 µM, range 25 µM-77.83 µM, range 2.45 µM-12.5 µM). Pre-BCR 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.

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.
treat relapsed patients, there is still a need to develop new and effective targeted therapies. Previous evidence has shown IL-7 as a fundamental cytokine for normal T-ALL cell viability and homeostasis, as well as an important determinant for the T-ALL cell viability and proliferation in vitro and in vivo. Several strategies have been explored for the treatment of T-ALL, and targeting Interleukin 7 receptor alpha-chain (IL-7Rα) and downstream IL-7 signaling offers a potentially effective therapeutic strategy for T-ALL. The IL-7Rα is recruited and concentrated into lipid rafts thereby amplifying its downstream signaling pathway. The IL-7/STAT5 signaling pathway mediates downregulation of pro-apoptotic signals on human T-cells such as BAD and BIM. Moreover, we have previously reported that a new compound named Pyrido[4,3-b]Quinoxaline (PyQ) has an anti-tumoral effect on Acute Myeloblastic Leukemia (AML). It strongly interacts with the plasma membrane of AML cells and affects the positioning of the protein tyrosine phosphatase CD45, which is usually organized in microdomains into lipid rafts floating on the cell surface, and is delocalized by PyQ.

**Aims:** The aim of this study was to assess the anti-tumoral effect of PyQ on T-ALL cells and to identify which signaling pathway is affected by the compound.

**Methods:** We have 2 models of human T-ALL which can be studied in vitro when cocultured with murine stromal MS5 cells and in vivo when transplanted into 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.

**Results:** In this study, we have shown that PyQ delocalizes the IL-7Rα away from lipid rafts from the 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 maintenance. 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 IC_{50}=5.7 ng/mL). For this work, T-ALL cells were co-cultured on 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 mice xenografted with T-ALL cells.

**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.
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. Post-transplant maintenance using tyrosine kinase inhibitors (TKIs) to reduce the relapse rate remains a subject of uncertainty, as data from prospective studies are limited.

Aims: To determine the impact of IM administration after HSCT on patient outcome and to assess the predictive value of minimal residual disease (MRD) analysis by qRT-PCR of BCR-ABL1 transcripts.

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, absence of infection, or immunosuppressive dose of IM was 600mg or 400mg recommended as starting dose. Primary endpoint was molecular or hematologic relapse, secondary endpoints included survival, DFS, severe toxicity and transplant-related mortality. All pts. were followed by frequent serial MRD analysis after HSCT. An interim analysis was reported previously. We here provide results of the final analysis of this trial, with long-term follow-up of up to 11 years after HSCT.

Results: 74 pts. were evaluable, 36 received prophylactic and 38 pts. pre-emptive IM. Median age was 41 y (18-89) and 44 y (19-68), respectively. Disease status at HSCT was CR1 (n=67), CR2 (n=5), n=1) (n=1), unknown (n=1). Most pts. received a PBS graft (n=61) and myeloablative TBI-based conditioning (n=65), 8 pts. underwent RIC with 2Gy or 4Gy TBI (n=6) or non-TBI RIC (n=2). Median time from HSCT to starting IM was 48d and 77d, respectively. IM dose was 600mg/d in 22% of pts., remaining pts. received 400mg. Treatment was prematurely discontinued in 56% and 59% of pts., median time to discontinuation was 92d (range, 0-111) and 71d (range, 0-786). 10y DFS of IM was 80.7% (95% CI: 75.1-85.8) and 71% with prophylactic and pre-emptive IM, respectively (p=ns). MRD levels were significantly predictive of relapse: BCR-ABL1/ABL1 (B/A) ratio ≥10-3 within 6 weeks prior to HSCT was associated with a higher cumulative incidence of relapse (CIR) (at 47.5% vs 10.6%, p=0.006) and inferior DFS (45% vs 79%, p=0.027) at 10y. B/A ratio ≤10-4 within 100d after HSCT was likewise associated with a higher CIR (21% vs 6%, p=0.018) and inferior DFS (55% vs 71% at 8y). An algorithm combining pre- and early (<100 days) post-transplant MRD levels (pre: ≥10-4; post: any positivity including below quantitative range) identified patients with a 60% vs 8.5% CIR at 10y.

Summary/Conclusions: Post-HSCT intervention with prophylactic or pre-emptive IM is associated with a low relapse risk and excellent long-term survival. A combination of pre- and post-transplant MRD analysis (10 deaths, 9 manufacturing failures, 3 adverse events [AEs], 4 pts. discontinued IM) is promising. 97 pts. received a single infusion of transduced CTL019 cells (median dose, 3.2×10^6 [range, 0.2-5.4×10^6] cells/kg). Median age was 12y (range, 3-25). During the first 8 wk after infusion, 98% of pts. experienced an AE of any grade (G), 82% experienced G3/4 AEs, and 72% experienced a serious AE (SAE). Common nonhematologic G3/4 AEs (≤10%) during the first 8 wk were cytokine release syndrome (CRS; ≤4%), hypotension (≤3%), decreased appetite (≤21%), increased AST (≤19%) and ALT (≤12%), hypoxia (≤16%), hypokalemia (≤13%), hypophosphatemia (≤11%), and pulmonary edema (≤10%). Rates of G3/4 AEs and SAEs decreased substantially >8 wk post infusion. 21 pts died post infusion: 16 (8.5%) from B-ALL (n=2), 320 days after infusion; n=14, >30 days); cerebral hemorrhage (n=1) and embryonic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days). Safety events were similar across pt subgroups based on age, sex, prior allogeneic stem cell transplant (alloSCT) (n=57), and Down syndrome (n=7). CRs were based on the IPSS scale, occurred in 81% of pts (Table 1). All CRS events occurred ≤8 wk post infusion. CRS was managed with supportive care, and 34% of pts were treated with anti-IL-6 agents. No deaths were attributed to CRS. Pts. with ≤50% bone marrow (BM) blasts at enrollment (n=68) were

Results: 123 pts were enrolled, 26 were not infused and not included in this analysis (10 deaths, 9 manufacturing failures, 3 adverse events [AEs], 4 pts. prematurely discontinued). 97 pts. received a single infusion of transduced CTL019 cells (median dose, 3.2×10^6 [range, 0.2-5.4×10^6] cells/kg). Median age was 12y (range, 3-25). During the first 8 wk after infusion, 98% of pts. experienced an AE of any grade (G), 82% experienced G3/4 AEs, and 72% experienced a serious AE (SAE). Common nonhematologic G3/4 AEs (≤10%) during the first 8 wk were cytokine release syndrome (CRS; ≤4%), hypotension (≤3%), decreased appetite (≤21%), increased AST (≤19%) and ALT (≤12%), hypoxia (≤16%), hypokalemia (≤13%), hypophosphatemia (≤11%), and pulmonary edema (≤10%). Rates of G3/4 AEs and SAEs decreased substantially >8 wk post infusion. 21 pts died post infusion: 16 (8.5%) from B-ALL (n=2), 320 days after infusion; n=14, >30 days); cerebral hemorrhage (n=1) and embryonic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days). Safety events were similar across pt subgroups based on age, sex, prior allogeneic stem cell transplant (alloSCT) (n=57), and Down syndrome (n=7). CRs were based on the IPSS scale, occurred in 81% of pts (Table 1). All CRS events occurred ≤8 wk post infusion. CRS was managed with supportive care, and 34% of pts were treated with anti-IL-6 agents. No deaths were attributed to CRS. Pts. with ≤50% bone marrow (BM) blasts at enrollment (n=68) were

Table 1.
twice as likely to develop G3/4 CRS than pts with <50% BM blasts (n=29) (53% vs 29%; P<0.0001). InO, more patients with diploid (≥20 metaphases) karyotype proceeded to stem cell transplant versus other cytogenetic subgroups. With InO, the duration of CR/CRi and OS did not differ according to whether ASCT was performed (P=0.5427). 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=0.7653). 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). Within 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.

Table 1.

<table>
<thead>
<tr>
<th>Cytogenetic Subgroup</th>
<th>PFS</th>
<th>OS</th>
<th>CR/CRi</th>
<th>MRD Negativity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid (≥20 metaphases)</td>
<td>100%</td>
<td>97.5%</td>
<td>97%</td>
<td>76%</td>
</tr>
<tr>
<td>MLL</td>
<td>97.5%</td>
<td>97%</td>
<td>97%</td>
<td>76%</td>
</tr>
<tr>
<td>Complex (&gt;5 abnormalities)</td>
<td>97%</td>
<td>97%</td>
<td>97%</td>
<td>76%</td>
</tr>
<tr>
<td>Other</td>
<td>97%</td>
<td>97%</td>
<td>97%</td>
<td>76%</td>
</tr>
</tbody>
</table>

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<0.0001), with good OS in both. 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 SCHEDULE AS SALVAGE THERAPY FOR RELAPSED/REFRACTORY ACUTE LYMPHOBlastic LEUKEMIA (R/R) IN ADULT PATIENTS


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Aims: To assess the activity and toxicity of the sequential clofarabine-cyclophosphamide regimen for adults with relapsed/refractory acute lymphoblastic leukemia.

Methods: An open-label, single-arm, phase II trial was conducted at six sites in Italy. Eligible patients aged 18-75 years with relapse or refractory AML or ALL were enrolled. The treatment consisted of a 5-day cycle of clofarabine 35 mg/m²/day, followed by cyclophosphamide 600 mg/m²/day. Two cycles were administered, with a 4-week interval between cycles.

Results: 15 patients were enrolled, with a median age of 52 years (range: 18-75). 14 patients (93%) had relapsed disease, and 1 patient (7%) had refractory disease. Complete response was achieved in 9 patients (60%) after 2 cycles of treatment. The median OS was 9 months (range: 1-124) and the median DFS was 6 months (range: 1-24). There were no grade 5 toxicities, and the most common grade 3/4 toxicities were febrile neutropenia (60%) and thrombocytopenia (40%). The overall response rate was 60%, with 0 CRs, 9 CRs, and 6 non-responders.

Summary/Conclusions: The sequential clofarabine-cyclophosphamide regimen showed acceptable activity and manageable toxicity in adults with relapsed/refractory AML or ALL. Further studies are needed to confirm these results and explore the potential benefits of this regimen in other settings.

Table 1: Summary of patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Median 52 (range 18-75)</td>
</tr>
<tr>
<td>Disease status</td>
<td>Relapsed 14 (93%), Refractory 1 (7%)</td>
</tr>
<tr>
<td>Response rate</td>
<td>60% (9 CRs, 6 non-responders)</td>
</tr>
</tbody>
</table>

P521
BLINATUMOMAB USE IN PEDIATRIC AND ADULT PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBlastic LEUKEMIA: AN OPEN-LABEL, MULTICENTER, EXPANDED ACCESS STUDY

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Aims: To evaluate the safety and efficacy of blinatumomab in adult and pediatric patients with relapsed/refractory B-precursor ALL enrolled in an expanded access study (NCT02187354).

Methods: Eligible patients (aged ≥18 years) with relapsed/refractory B-precursor ALL were treated with blinatumomab at a dose of 0.8 mg/m²/day for 3 weeks followed by 2 weeks off. The primary endpoint was the proportion of patients achieving complete response (CR) with or without CR with incomplete recovery (CRi) after 2-4 cycles of treatment. Efficacy endpoints included overall response rate (ORR) and duration of response.

Results: 21 patients were enrolled, with a median age of 24 years (range: 18-64). 10 patients achieved CR (45%) and 6 patients achieved CRi (28%). The median duration of response was 12.4 months (range: 2-50). The most common grade 3/4 adverse events were pyrexia (71%), cytokine release syndrome (23%), and neutropenia (21%). No grade 5 adverse events were reported.

Summary/Conclusions: Blinatumomab showed acceptable safety and efficacy in adult and pediatric patients with relapsed/refractory B-precursor ALL. Further studies are needed to confirm these results and explore the potential benefits of this regimen in other settings.
KTE-C19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY IN ADULTS WITH HIGH-BURDEN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL): UPDATED RESULTS FROM PHASE 1/2 OF ZUMA-3


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Background: The incidence of acute lymphoblastic leukemia (ALL) is increasing, with nearly 6600 new diagnoses expected in 2016, of which >40% will affect children under the age of 10 years. Adult patients (pts) with B-ALL show high-risk disease biology, high rates of relapse, and poor survival (J Clin Oncol 2011;29:532; Blood 2012;119:34). Promising results have been observed with KTE-C19, an anti-CD19 chimeric antigen receptor (CAR) T cell therapy, in refractory, aggressive non-Hodgkin lymphoma (Blood 2016;128:LBA-6), and suggest an opportunity to improve outcomes in ALL. Here, we present updated results from phase 1 portion of ZUMA-3, a multi-center study of KTE-C19 in pts with high tumor burden ALL.

Aims: The goal of this study is to assess safety and efficacy of KTE-C19 in adult pts with relapsed/refractory ALL who have high disease burden.

Methods: Eligible pts were ≥18 years of age with relapsed/refractory ALL (Ph+ pts eligible), ≥25% bone marrow lymphoblasts, adequate organ function, and Eastern Cooperative Oncology Group status 0-1. Pts received 1 or 2 × 10⁶ CAR T cells/kg after conditioning with cyclophosphamide and fludarabine. The primary endpoint of phase 1 was incidence of dose-limiting toxicity (DLT). Secondary endpoints were efficacy outcomes of KTE-C19, including complete response (CR) rates and biomarker associations.

Results: As of Nov 1, 2016, 11 pts were enrolled, and 10 were treated with KTE-C19. One pt had a serious adverse event prior to dosing and was not treated. KTE-C19 was successfully manufactured in a centralized facility for all pts across a broad range of baseline absolute lymphocyte counts in 6 days, with a turnaround time of ~2 weeks. Pts were 60% men, with 1-4 prior lines of 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 cytopenias (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 cut-off). 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 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.

Impact: KTE-C19 demonstrates promising efficacy and manageable safety in a phase 1/2 study of pts with high burden ALL. The results support phase 2 expansion and enhanced safety measures to further improve outcomes in this difficult-to-treat ALL population.

P524

EXPOSURE-ADJUSTED ADVERSE EVENTS COMPARING BLINATUMOMAB 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 bispecific 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-precur sor acute lymphoblastic leukemia (ALL) in a randomized phase 3 study (N Engl J Med 2017;376:836-847).

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 5 induction cycles (9 µg/day on days 1−7 of cycle 1, 28 µg/day thereafter). Up to four maintenance cycles (1 of 4 predefined regimens). Blinatumomab was dosed by continuous intravenous infusion (4 weeks on/2 weeks off) 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) were lower for blinatumomab than for SOC after adjusting for varying treatment exposure times for a more comprehensive evaluation of safety and tolerability.

Impact: Blinatumomab was associated with a manageable safety profile. Based on these results, exposure-adjusted event rates can be used in clinical decision making or to aid in prioritization of future research.
Table 1.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Standard of Care</th>
<th>High Intensity</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total remission, years</td>
<td>5</td>
<td>5</td>
<td>0.23</td>
</tr>
<tr>
<td>Number of Events</td>
<td>129</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Exposure-adjusted Event Rate</td>
<td>100%</td>
<td>100%</td>
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</tr>
<tr>
<td>Number of Events</td>
<td>433</td>
<td>491</td>
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<tr>
<td>Exposure-adjusted Event Rate</td>
<td>100%</td>
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<td></td>
</tr>
</tbody>
</table>

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.

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 MULTI-DOSE REGIMENS

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1ImmunoGen, Waltham, United States

Background: Antibody-drug conjugates (ADCs) targeting CD33 are promising therapeutics 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 in 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-sulfobutane (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 Ti in human AML xenografts. ADCs with cleavable and non-cleavable linkers were evaluated for cytotoxicity on MDR-positive and -negative AML cell lines, for tolerability in mice and Ti 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 payload 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 IGN payload class. Both versions had comparable IC50 on human AML cell lines as free drugs (12-260 vs. 5-77 PM) and as CD33-targeting ADCs (0.7 vs. 0.5 PM). However, in vivo, the CD33-targeting DNA alkylating ADC had a 50%-higher MTD (maximally tolerated dose) in mice and 50% larger Ti in AML xenograft models (MTD 950 vs. 180 µg/kg, by payload, Ti 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, Ti), 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 (mice, 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 a 90% increased life span, and was well-tolerated and highly active in repeat dosing regimens (10 or 30 µg/kg, qx 3 and q3dx 3) in a HL60 AML xenograft model. Similarly, in a MV4-11 xenograft model, a single dose of IMGN779 was highly active in single-dose (5 µg/kg, qx 3 or q4dx 3) and 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 regimens, 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 PAF1 TO CLEAR POLYCOMB-INDUCED TRANSCRIPTIONAL REPRESSION

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Background: In mixed lineage leukemia ENL is frequently found juxtaposed to MLL creating MLL-ENL fusion proteins that initiate leukemogenic transfor- mation. Interestingly, murine ENL has also been shown to have oncogenic activity, a pediatric nephroblastoma. In its wild-type configuration ENL serves as scaf-olding factor in protein complexes that stimulate transcriptional elongation but, paradoxically, it also co-purifies with polycomb repressive complex 1 (PRC1).
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) and a chromatin rearrangement that enhances the ability to bind PAF1. This led to a significant and dose-dependent increase in mitochondrial-produced superoxides—a specific type of ROS. Moreover, we found that enforced expression of PFKcC can protect AML cells from lethal effects of superoxide-inducing agents 2-thienyltrifluoroacetone and Antimycin A. To identify potential ROS-regulatory enzymes downstream of PFKcC, we performed whole cell proteomics and found that the mitochondrial superoxide-neutralizing enzyme SOD2 is decreased in AML cells depleted of PFKcC. Similar to PFKcC inhibition, we also observed gene expression changes in 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 PFKcC inhibition confirming that PFKcC supports AML pathophysiology by maintaining mitochondrial redox homeostasis. Summary/Conclusions: Our results indicate that PFKcC 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 Ptpn11, 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.

Methods: Aims: Inactivation of polycomb by ENL was accompanied by ubiquitination of histone H2B, the hallmark activity of PAF1 allied enzymes. On a global level, high resolution RNA-Seq demonstrated that MLL-ENL target genes stood out with a supraphysiological accumulation of H2B ubiquitination and H2B ubiquitination of Hox9a 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 ENL silencing, transcriptional interaction with PAF1 and thus perturbed proper silencing. This effect was intensified in a 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 MITOCNDRIAL 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 reactive oxygen species (ROS) levels, which are a class of free radical molecules. Though ROS are by-products of several cellular processes, in excess, they can damage DNA and destroy organelles, resulting in the acquisition of genetic mutations or cell death. As a result, ROS homeostasis is tightly regulated by an array of molecular pathways. Although ROS is elevated in AML cells, the role of ROS and the identity of its regulators remain largely unknown. Here we report that the serine/threonine kinase, PKCε regulates the ROS-neutralizing enzyme SOD2 to support mitochondrial redox homeostasis and AML progression.

Aims: The goal of this study was to identify and subsequently assess how targeting key ROS-regulatory pathways impacts AML biology. Methods: Loss-of-function studies for PKCε and SOD2 were performed with recombinant lentiviruses expressing gene-targeting shRNAs. Recombinant retroviruses expressing either PKCε or SOD2/Catalase were used for gain-of-fusion assays. Cytoplasmic and mitochondrial superoxides and peroxides were measured using redox-sensitive GFP (roGFP) probes followed by flow cytometric analysis. Mitochondrial superoxides were also assessed by flow cytometric analysis of MitoSox stained cells. Proteomic analysis was achieved using nano LC-MS/MS. Annexin-V staining was analyzed by flow cytometry to measure apoptosis and in vitro and in vivo survival assays were performed to evaluate the ability of mice lacking PKCε or its FACS-based purification of shRNA-expressing cells followed either by: 1) growth in cytokine-enriched media or 2) transplantation into syngenic mice for cytometric analysis. Mitochondrial superoxides were also assessed by flow cytometric assays. Cytoplasmic and mitochondrial superoxides and peroxides...
GENE MUTATIONS AND THE SENSITIVITY OF LEUKEMIC CELLS TO KINASE INHIBITION DEPENDS IN NON-AND INHIBITORS REMAINS POORLY DEFINED AND THESE HAVE NOT BEEN TRANSLATED INTO EFFECTIVE THERAPIES. Thus, the integrative analysis of genetic mutations on the sensitivity of primary AML to kinase inhibition 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 regulation, 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. In silico data analysis and Chi-sq experiments together with series of knockdown and restore experiments identified BCL11A and TRIM24 as critical mediators of p53 pathway activation in RUNX1-inhibited AML cells.

Although RUNX1-depleted AML cells exhibited drastically slowed proliferation rate, a small sub-population of leukemia cells retained the proliferation potential even after the 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 family 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 patients with xenograft mice models of AML, acute lymphoblastic leukemia, lung and gastric cancers, extending their overall survival periods in vivo. Since RUNX family takes part in diverse physiologic functions not only in AML cells but also in normal hematopoietic cells and in various other vital organs 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 counterparts, and we believe that this gap offers pharmacological window to be targeted efficiently and exerted excellent efficacy against xenograft mice models of AML, acute lymphoblastic leukemia, lung and gastric 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


**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 multimomics 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, c-Abl, FLT3/PKC 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 CD9+ and CD9- groups. Remarkably, the M4-like and CD9+ groups representing the differentiated cases, as well as the M1-like and CD9- 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 estimated that differentiated groups presented an enriched activity for PAK, MEK, ERK or PCK. 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 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.

**P533**

CLINICAL OUTCOMES OF TET2 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS HARBORING CEGBPA MUTATIONS: A STUDY OF THE AML STUDY GROUP (AMLSG)

Background: The proper differentiation of hematopoietic stem cells (HSCs) is regulated by transcription factors. Growth factor independence 1b (Gfi1b) is a repressing transcription factor regulating quiescence of HSCs and the proper emergence and maturation of erythrocytes and platelets.

Aims: Aim of the study was to identify i) do different level of Gfi1b influence onset and development of MDS and AML in human patients II) how does Gfi1b act in MDS/AML development on a molecular level.

Methods: We correlated Gfi1b expression level in blast cells of patients with MDS and AML with the overall disease course. To get a better insight how does Gfi1b expression level influence clonal evolution we used three different murine models of human AML with expression of different oncogenes (NUP98/HOXD13, MLL-AF9 and expression of a mutated K-Ras). In these models we either downregulated or conditionally knocked out Gfi1b expression. Finally, we performed ChiP Seq analysis as well as whole genome expression arrays to study the molecular functions of Gfi1b in AML development.

Results: Low expression or absence of Gfi1b expression was associated with an inferior outcome with regard to overall-survival as well as event-free survival of MDS/AML patients. Using the above murine models of MDS/AML, loss or low expression of Gfi1b accelerated AML development. Additionally we could show that expression of Gfi1b is able to enhance number of functional committed stem cells. It is well known that Gfi1b has a function to recruit histone modifying enzymes to induce among other deacetylation of H3K9. ChIP seq data of Gfi1b deficient leukemic cells revealed that loss of Gfi1b led to a higher H3K9 acetylation of a target of target genes, among them a number of oncogenes.

Summary/Conclusions: Gfi1b acts as a tumor 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 (AML) AT M1 UNDER TREATMENT WITH AND WITHOUT DASATINIB

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Background: Recent next-generation sequencing (NGS) studies have improved our understanding of the genomic landscape of CBF-AML (Fabel et al. Nat Genet 2016; Duployez et al. Blood 2016). While these studies have mainly focused on the genetic differences between inv(16)- and t(8;21)-AML, the role of additional mutations required for disease evolution is poorly defined. Mutations affecting signaling genes, such as KIT and NRAS are known to be among the most common oncogenic drivers in CBF-AML, however their impact at relapse remains unclear.

Aims: To characterize clonal evolution in paired samples obtained at diagnosis, as well as during treatment with dasatinib with data from 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 [mi(16), n=24; t(8;21), n=12] using paired-end sequencing (read length 100 bp) on an Illumina HiSeq platform. Library preparation was done with the Nextera® Rapid Capture Exome kit following manufacturer’s instructions. All patients were treated within one of five trials of the German-Austrian Study Group (AMLSG). In two of the trials (AMLSG 11-08, NCT00850382; AMLSG 21-13, NCT02013648) patients received intensive chemotherapy in combination with the multi-kinase inhibitor dasatinib.

Results: The mean WES coverage was 133x. Mutations and indels were called with a threshold >10% variant allele frequency (VAF) after filtering for SNPs and sequencing artefacts. In sum, we identified 587 variants in 430 genes. At
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, p38a and p38β, demonstrated that only p38β was able to set RED 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 cicloheximide 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 the absence of p38β, 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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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 possession 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.
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 myelodysplasia. 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 types of AML and MDS. 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 baits were also designed for a total of 1158 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 targeted-capture sequencing in AEL, as compared to 12.2 and 2.9 mutations (P>0.05) in other 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%), MLL (12%), SMCA1 (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 among the top targets of mutations, including SRSF2 (12%), U2AF (4.8%), WT1 (15%), TET2 (19%) and ID1 (2.1%). TP53 mutations were mutually exclusive with cohesin mutations (p<0.01) and those in epigenetic regulators in the majority of AML cases (98%). Furthermore, a total of 84 AEL cases were screened for mutations in 67 driver genes from other types of AML and MDS. In an association model, which takes all other known subsets of AML into account, other mutations were significantly more frequent in AEL and secondary AEL. Summary/Conclusions: MLL-PTD AML molecular subsets. EXPLORING THE IMPACT OF LOSS OF FUNCTION STAG2 MUTATIONS ON CHROMATIN ARCHITECTURE IN MDS/AML Background: The Cohesin complex is an evolutionarily conserved multimembrane protein complex consisting of SMCA1, SMCD3, SCMA3, STAG1, STAG2, and LSC1. The complex plays pivotal roles within mitosis and sister chromatid cohesion however, substantial data exists elucidating roles for the complex within the DNA damage response, homologous recombination and long-range interaction between cis regulatory elements of the genome. Within hematological malignancies, upwards of 20% of patients diagnosed with either Acute Myeloid Leukemia (AML), Myelodysplastic syndrome (MDS) or Myeloproliferative neoplasm (MPN) have been shown to harbour mutations within the Cohesin complex, with many more showing significantly reduced expression of core complex members. Aims: To explore the impact of a loss of function STAG2 mutation on the chromatin architecture within a 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 STAG1 and STAG2 samples. 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 matched 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. In addition, histone mark profiling identified wide spread 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 ATAC-Seq coincides with H3K27ac-sold region and a decrease in transcription factor occupancy. An enrichment for transcription factors relevant for myeloid transcriptional programmes was observed. This increase in enrichment aids in the suggestion that the impact of mutated/alternate cohesin complex function relates directly to the specific cell type and maturation stage at which it occurs. Summary/Conclusions: This research into the aberrant and non-canonical consequences of loss 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.
leukaemic cell lines; KG1 (CD123 +, CD34+, CD33+) [Fig:1a], Kasumi-1 were transduced (MOI 1:5) into peripheral blood mononuclear cells (PBMCs) the same assay conditions. (CD28 for CD123 and evaluated the cytotoxic effect of different co-stimulatory domains expressed on AML blasts and leukaemia stem cells (LSC) compared to normal tumour-associated antigens are emerging to be an effective form of induction treatment. Chimeric antigen receptor (CARs) T cells specific for AML biology, development of novel therapies has been limited with 43% relapse rate and 18% of patients never attaining clinical remission (CR) with frontline AML. 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 AML.

**Background:** Mutations involving the MLL gene at 11q23 are found in 10% of adult and 18% of childhood acute myeloid leukaemia (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-FFps). MLL-AF9 is the most common MLL-FF in AML. Despite much progress in the overall management of AML, patients carrying MLL-arrangements 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 leukaemia 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 leukaemia and the network it regulates, we maybe 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 leukaemia.

**Methods:** We performed genome-wide MLL, MLL-AF9, H3K27ac, H3K4me3 and H3K4me1 chromatin immunoprecipitation (CirP) sequencing (ChIP-seq) and Assay for Transposase-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 knocked-down 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 leukaemia, suggesting a molecular rational for using BCL2 inhibitors in MLL-AF9 leukaemia therapy.

**P542**

**TARGETED COMBINATION THERAPY WITH CDK4/6 INHIBITOR PALBOCICLIB IN AML**

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**Background:** Acute myeloid leukaemia (AML) is a clonal hematologic disorder marked by clinical and biological heterogeneity. AML remains incurable for a significant proportion of adult patients while no therapeutic option exists for patients with relapsed and refractory AML. Mutations of the fms-like tyrosine kinase 3 (FLT3) gene are among the most frequent events in AML and usually involve internal tandem duplication (ITD) of the juxtamembrane domain coding region or point mutations of the tyrosine kinase domain. There have been considerable efforts to develop FLT3 tyrosine kinase inhibitors (TKI). The clinical impact of FLT3-TKI has been limited as resistant clones have emerged rapidly.

In conclusion, we demonstrate the importance of the scFv on CAR T cell cytotoxicity and have constructed and validated the efficacy of a dual targeting CAR vector in the context of AML.
We have recently discovered that FLT3-ITD AML cells are highly sensitive to the FDA-approved SFK inhibitor, AP23454 (LY3176159) and a CDK4/6 inhibitor, abemaciclib (X010). The effect is ascribed to the transcriptional activity of CDK6 on FLT3 and PIM1 - a feature not shared by CDK4.

**Aims:** FLT3-TK1 treatment provides short-term disease control but relapse invariably occurs within months. Acquired resistance on FLT3-D835Y tyrosine kinase domain activation is a major concern. Thus, our focus is to investigate the potential of palbociclib treatment in FLT3-D835Y+ cells and to identify critical downstream effectors of CDK6 to open a novel, clinically applicable therapeutic window.

**Methods:** Ba/F3 cells transformed with FLT3-D835Y were exposed to single agents or drug combination by the CellTiter-Glo ATP-based assay and FACS stainings after 3 days of treatment. Validation was performed by in vivo xenograft models and by studies with primary human FLT3-D835Y AML biopsies.

**Results:** Palbociclib impaired the viability of murine Ba/F3 cells with FLT3-D835Y, as expected, which led to apoptosis. The effect on FLT3-D835Y patient samples and to xenograft models, where palbociclib treatment effectively repressed FLT3-D835Y driven tumor formation in vivo at clinically relevant concentrations. Besides FLT3 itself, which is regulated by CDK6, transcriptional targets of CDK6 in AML included Aurora kinases (AURK) and AKT. Thus CDK6 inhibitor, such as palbociclib, is capable of inducing AML cell death, two signalling nodes critical for survival of tumor cells. Dual targeting with palbociclib and AURK or AKT inhibitors resulted in synergistic cytotoxicity.

**Summary/Conclusions:** Palbociclib represents a viable therapeutic option for use in treatment of resistant clones in FLT3-D835Y+ AML. Inhibitory effects are not limited to cell viability but as well by transcriptional activity of CDK6 on important signalling pathways including Aurora kinases and AKT. Our findings provide the basis for the design of synergistic combination therapies with a CDK4/6 inhibitor which could be readily translated to patients with AML.

P543

**CANNABINOIDS DERIVATIVES MODIFY THE PATTERN OF SPHINGOLIPIDS IN ACUTE MYELOID LEUKEMIA CELLS AND PRODUCE A POTENT ANTI-LEUKEMIC EFFECT**

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**Background:** Endocannabinoid system is a set of ligands, receptors and endogenous enzymes which modulate a variety of physiological effects. There are two well-characterized cannabinoid receptors, CB1 (mainly expressed in Central Nervous System) and CB2 (mainly in hematopoietic cells). Here, we tested the effect of the cannabinoid WIN-55 212-2 in acute myeloid leukemia (AML) in vitro and in vivo and studied the molecular signaling pathways involved in this effect, specially the role of sphingolipids. Moreover, we synthesized a new family of twelve cannabinoids that are specific to CB2 receptor.

**Aims:** Development of new compounds derived from cannabinoids with CB2 selectivity and evaluation of their anti-tumor effect in AML in vitro and in vivo. To deepen in the knowledge of lipid metabolism in AML.

**Methods:** For the design and synthesis of new cannabinoids, computational techniques of docking, analytical and spectroscopic techniques such as mass spectrometry (MS) were used. To assess the anti-leukemia effect of the different cannabinoids, we analyzed cell viability by MTT and flow cytometry using six techniques of docking, analytical and spectroscopic techniques such as mass spectrometry and immunohistochemistry assays to determine the expression of different proteins to elucidate the molecular signaling pathways involved in the effect of these drugs. Moreover, we synthesized a new family of twelve cannabinoids that are specific to CB2 receptors.

**Results:** Cannabinoids induced a potent proapoptotic effect on AML cell lines and primary leukemic cells, which was not observed in normal HPC and lymphocytes from healthy donors. Fragmentation of PARP and activation of caspases 2, 3, 8 and 9 were confirmed by Western Blot. The proapoptotic effect of cannabinoids on AML cells was abolished upon co-culture with either CB2 receptor antagonists or with pancaspase inhibitors. Other proteins involved in this proapoptotic effect of cannabinoids were p-ERK, p-PI3K and p-JNK. Also, studies on p-PERK, p-IRE1 and CHOP confirmed an increased endoplasmic reticulum stress upon exposure to cannabinoids. Also we confirmed a very early mitochondrial damage in leukemic cells which was not observed in normal hematopoietic progenitor cells. Remarkably, we observed significant differences in the amounts of certain sphingolipids in untreated versus treated leukemic cells. More specifically in ceramide C16:0, C18:0 and C18:1. Also we observed a significantly increased survival among mice treated with WIN-55 cannabinoid as compared to both the control group and the group treated with ARA-C and we confirmed that cannabinoids did not affect the viability of the different population hematopoietic progenitors and, moreover, an increased platelet count was observed in treated mice.

**Figure 1.**

**Summary/Conclusions:** Our findings indicate that cannabinoids display a highly selective proapoptotic effect against leukemic cells. Several pathways are involved in this effect, the modification of the sphingolipids pattern playing a main role.

P544

**PROFILING THE MUTATIONAL LANDSCAPE OF ACUTE MYELOID LEUKEMIA AT RELAPSE AFTER CHEMOTHERAPY AND ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background:** Acute Myeloid Leukemia (AML) is a clinically and biologically heterogeneous disease that is known to dynamically evolve over time. Unraveling its mutational profile may provide relevant insights into the inception, propagation, and recurrence of the disease, and deliver new rationales for precision medicine approaches: still, whereas a comprehensive description of AML mutations at disease presentation is now available thanks to large-scale studies, a satisfying genomic characterization of AML at relapse, particularly after allogeneic stem-cell transplantation (allo-HSCT), is still needed.

**Aims:** To characterize the genetic profile of relapsed AML, highlighting the evolutionary trajectories in the two different settings of relapse after chemotherapy (CT) and after allo-HSCT.

**Methods:** For our custom-designed targeted Next Generation Sequencing panel we took advantage of the HaloPlex High Sensitivity (HS) technology, allowing a more precise definition of mutations and clonal architecture through a molecular barcoding system. We included in our panel 192 genes and miRNAs known to be involved in the pathogenesis of myeloid malignancies (n=112), in the DNA damage response (n=50), or in immune-related processes (n=50). 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:** We sequenced a total of 138 AML samples, including 79 diagnoses, 36 relapses after CT and 23 relapses after allo-HSCT. 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 definable as oncogenic), with a median of 3 oncogenic mutations per patient (range 0-4), and mutation frequencies in line with the largest published dataset (Papaemmanuil, N Engl J Med, 2016; r²=0.83). In relapses after CT and after allo-HSCT the median number of oncogenic mutations per patient was 3 (range 0-4) and 2 (range 0-6), respectively. Comparing mutation frequencies at relapse
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.

P545
Abstract withdrawn.
22nd Congress of the European Hematology Association
analyses. 81% of pts had de novo AML, 15% secondary AML, 3% therapyrelated 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 mutational
hotspots or the entire coding sequence of the genes by multiplexed amplicon
sequencing (Agilent Technologies, mean target coverage of 460 x). We studied
associations between cytogenetics, gene mutations and other potential prognostic factors which might influence the clinical outcome.
Results: The median age in the total cohort was 76 years (y) (range: 75-86 y).
44% of pts reached complete remission (CR) and 4% CR with incomplete blood
count recovery (CRi). The median overall survival (OS) was 6 months with a
3-year OS of 21%. According to the ELN 2010 classification, 20% of pts were
in the favorable, 39% and 25% in the intermediate I or II group, respectively,
and 15% in the adverse group (ELN 2017 data will be presented at the meeting). Pts in the favorable and intermediate I/II groups had significantly longer
OS compared to the adverse group (median OS 6.3 vs 1.2 months, p=.002,
Figure). Likewise, pts in the favorable and intermediate MRC cytogenetic risk
categories had longer OS than those in the adverse category (median OS 6.5
vs 1.2 months, p=.001). By targeted sequencing, we detected 622 leukemiaassociated mutations in 66 genes. The median number of mutated genes per
patient was four. The most commonly mutated genes were TET2 (42%),
DNMT3A (35%), NPM1 (32%), SRSF2 (25%) and ASXL1 (21%). Both NPM1
or EZH2 (5%) mutated pts showed a non-significant trend towards longer OS
(NPM1: p=.09; EZH2: p=.065). FLT3-ITD mutations were identified in 29 pts
(19%), but had no impact on OS (p=.297). The NPM1mutated/FLT3-ITDnegative
genotype also did not associate with OS. Notably, none of the 13 IDH1 mutated
pts (9%; all within the ELN favorable/intermediate groups) reached CR, and
consequently the OS in this group was significantly shorter than for IDH1 wildtype pts (p<.001; Figure). The positive impact of mutated NPM1 on OS was
reversed when it co-occurred with IDH1 mutations (p=.014).
Summary/Conclusions: Among very old (≥75 y), intensively treated AML pts,
adverse-risk cytogenetics predict inferior survival. On the other hand, 3-year
OS was 24% for MRC/ELN favorable and intermediate-risk pts, suggesting
that even in this age group, selected pts without medical contraindications benefit from intensive induction chemotherapy. The spectrum of driver gene mutations in elderly pts differs from that in younger pts. While NPM1 and FLT3-ITD
mutations had no significant impact on OS in intensively treated pts aged ≥75
y, our data imply IDH1 mutations as a novel marker for chemorefractory disease
and inferior prognosis in this age group.

Results: To date, 47 pts have enrolled (Ph 1=19; Ph 2=28 of planned 47). The
recommended Ph 2 dose is 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). Common Gr 3/4 AEs were febrile neutropenia (36%), sepsis
(26%), bacteremia (13%), hypoxia (13%). Oral mucositis (≥Gr 2) 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
(CR/CRi) ratio was >2.75 (Estey, Blood 1996). 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 ligand in this population suggests relevance of the target and is predictive
of response.

P547

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.
Aims: BST-236 (Astarabine) is a new 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 medically unfit or older adults. The aim of this study was to evaluate the
safety and optimal dose of BST-236 in refractory/relapsed or newly-diagnosed
AML patients unfit for standard induction therapy.
Methods: A Phase I/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
patients, median age 64 (27-81), and 12 newly-diagnosed patients, unfit for
standard chemotherapy (7 secondary AML; 5 de novo AML/ALL), 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 BST236, all “on-target” hematological toxicity events or bacterial infections derived
from it. No neurological or grade >2 typical cytarabine events such as gastrointestinal, mucositis, or alopecia 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 responding (CR+PR) and of the CR patients was 7 and 10 months,
respectively. 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.
Summary/Conclusions: BST-236 is safe and very well tolerated, enabling
delivery of high dose cytarabine to older and unfit patients, resulting in overall
response and CR rates of 50% and 33%, respectively, and a 3-fold increase in
median OS of the responding compared to the non-responding newly-diagnosed patients. Notably, 67% of the responding patients had secondary AML,

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
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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; Winkler ASH 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 (R/R) AML.
Methods: A Phase (Ph) 1 trial in pts with R/R AML escalated GMI-1271 across
pharmacologically active doses from 5-20mg/kg combined with MEC to evaluate safety, tolerability and anti-leukemia activity. MEC consisted of mitoxantrone 10mg/m2/d, etoposide 100mg/m2/d, and cytarabine 1000mg/m2/d IV
for 5 days; supportive care was given as per institutional guidelines. Dose limiting toxicity (DLT) was defined as either persistent neutropenia and/or thrombocytopenia beyond day 42 in the absence of disease or any Grade 3 (Gr3)
non-hematologic toxicity that did not resolve to Gr2 by day 42. After confirming
safety and tolerability, a Ph 2 study of GMI-1271 at 10mg/kg plus MEC salvage
chemotherapy was initiated; responders to salvage could receive consolidation
with GMI-1271 plus reduced dose MEC (4 days). GMI-1271 was given 24 hrs
prior, then every 12 hrs during and for 48 hrs post induction/consolidation. Eligible pts had an ECOG score 0-2, received ≤2 prior inductions, had WBC <20K
(<40K after 2 dose levels), no active CNS disease, and adequate renal/hepatic
function. Baseline E-sel ligand expression on leukemic blasts in the bone marrow (CD45/SSC by flow) was assessed, as were plasma levels of soluble Esel (sE-sel).

210 | haematologica | 2017; 102(s2)

P548

BST 236, A NOVEL CYTARABINE PRO-DRUG ALLOW, FOR THE FIRST
TIME, THE DELIVERY OF HIGH CYTARABINE DOSES FOR OLDER OR
UNFIT PATIENTS WITH ACUTE LEUKEMIA. RESULTS OF AN ONGOING
PHASE I/IIA STUDY
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refractory to hypomethylating agents. To the best of our knowledge, this is the only experimental drug permitting high-dose cytarabine, considered a cornerstone of leukemia therapy, to be given to a population of patients that currently do not have this option. A Phase II study is planned to confirm these encouraging results.

**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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Background: Fusion 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 transcript.

Methods: 102 cases with myeloid malignancies harboring 105 translocations identified by chromosome banding analysis were selected. Recurrent fusion genes had been confirmed by FISH and/or RT-PCR. In cases with suspected novel fusions the rearrangement of one partner gene had been confirmed by FISH. The following recurrent rearrangements identified by standard diagnostic procedures were present: PML-RARA (n=11), RUNX1-RUNX1T1 (n=7), CSF3R-MYH11 (n=3), KMT2A-ELL (n=4), KMT2A-MLLT4 (n=4), KMT2A-MLLT10 (n=3), KMT2A-MLLT3 (n=2), BCRL1-ABL1 (n=3), NUP98-NSD1 (n=3), DEK-NUP214 (n=1), and KAT6A-CREBBP (n=1). Further, cases harboring MLLT4-KMT2A (n=14), RUNX1 (n=21), ETV6 (n=10), PDGFRB (n=10), RARA (n=2), 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 ~50ng RNA extracted from fresh/frozen samples. Sequencing was performed on the NextSeq instrument (Illumina) and analysis with the RNA-Seq Alignment 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 diagnostic, 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), ITI1R2, FLNC, ASXL2, DPO1B, MAML1 and ARHGEF12. Seven different partner genes were identified in RUNX1 translocations: PLAG1 (n=2), PRDM16, MECOM, ZFP2M, MAN1A2, NAM2T and KIAA1549L. Five different partner genes were identified in ETV6 rearranged cases: ABL1, CCDC126, ERG, FOXX1 and CFLAR-AS1. Most strikingly was the identification of the ETV6-ABL1 fusion, which could not be suspected by cytogenetics as the S ETV6 FISH signal was located on chromosome 17. In 710 PDGFRB rearranged cases the partners were identified. These were WDR4, CCDC88C, MPRIP, TNIP1, TPR, NFA and ZBTB11. Further the following fusions were found: NPM1-RP3P10, NPM1-SETBP1, NUP98-ING3, IRF2BP1-RARA, and ZBTB16-RARA. Thus, RNA sequencing identified 39 fusion transcripts which standard diagnostics had not identified; 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.

**P550**

COMPREHENSIVE MOLECULAR ANALYSIS OF ADULT MIXED PHENOTYPE ACUTE LEUKEMIA (MPAL)

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Aims: To clarify the underlying pathogenesis of MPAL and provide clue for future personalized therapy in MPAL, we performed comprehensive molecular characterization of adult MPAL.

Methods: We studied 31 patients with adult MPAL (median age 53) that met 2008 WHO classification criteria. Standard remission induction chemotherapy was performed based on 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 cases of which pre-treatment samples were sequenced 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, 16 (52%) had MLL and 7 (23%) had TET2 mutations. 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 mutations than B-ALL (median 0-4, P=0.01). In 42/45 (93%) cases with a recurrent rearrangement identified by FISH and/or RT-PCR, RNA-Seq Alignment App (BaseSpace Sequence Hub) using Star for Alignment and Manta for gene fusion calling with default parameters (Illumina).

Mutational analysis showed 39 fusion 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 FOXP1-DNAJC15, FLI1-IFT46, and ITPR2-ARID5B. 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, copy number variations, and gene expressions. Therapy for MPAL may need to be personalized based on genomic profiles.

**P551**

THE EFFECTS OF EARLY INTENSIFIED INDUCTION CHEMOTHERAPY IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA COMPARED TO STANDARD ANTHRACYCLINE PLUS CYTARABINE 3+7 CHEMOTHERAPY

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Background: Standard remission induction chemotherapy for acute myeloid leukemia (AML) which consists of anthracycline for 3 days plus cytarabine for 7 days was first introduced in 1970’s and has been used for a long time. Several modification or intensification for this conventional regimen did not prove the effect for higher complete remission (CR) rate or lower relapse rate which led to superior overall survival (OS) rate.

Aims: We tried to find out possible benefit of early intensification of standard induction chemotherapy in adult AML patients.

Methods: This retrospective study enrolled 1195 adult AML patients from 2002 to 2013. All patients were initially treated with idarubicin (12mg/m2) plus cytarabine (100mg/m2) or BHAC (300mg/m2) induction chemotherapy (3+7), and among them, 731 (61.2%) patients received additional early intensification using cytarabine 3 days (3+10, n=363) or anthracycline 2 days plus cytarabine (3+9, n=368). Demographics were compared between the two groups. Hematologic response were compared between the two groups. Hematologic response rates, relapse rates, overall survival (OS) and disease-free survival (DFS) were compared between the two groups.

Results: The median age at diagnosis was 65 (range: 19-84) years. The median follow-up time was 24 months (range: 1-130). There was no significant difference in age, sex ratio, and FAB subtype distribution. Most of patients were who were in de novo AML (93.9%). In the group of 3+7 chemotherapy, the median OS and DFS were 50.4 months and 21.9 months, respectively. In the group of 3+10 chemotherapy, the median OS and DFS were 59.2 months and 27.7 months, respectively.

Conclusion: Early intensification with anthracycline and cytarabine improved hematologic response rates, OS, and DFS. In conclusion, early intensification of induction chemotherapy for acute myeloid leukemia (AML) is a feasible and effective approach.
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 sub-group) and larger proportion of t(8;21) (n=102 [27.7%] vs 73 [3.1%] vs 3+10 [12.6%], P<0.001). Also, initial GM blast counts were higher in two intensified groups (73.3% in 3+10 and 70.1% in 3+10) compared to 3+7 sub-group (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% in 3+10 and 15.7% in 3+10 vs 6.3% in 3+7, P=0.038). CR rate after induction was higher 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 younger patients younger than 55 years (4% vs 9%, 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 groups 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).

**Table 1.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Post-induction early death/ relapse (%)</th>
<th>5 years OS (%)</th>
<th>5 years CR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>21.5%</td>
<td>79.7%</td>
<td>70.1%</td>
</tr>
<tr>
<td>Intensified</td>
<td>27.8%</td>
<td>68.3%</td>
<td>55.0%</td>
</tr>
</tbody>
</table>

**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 FLT clones. Previously available PCR-based tests only checked for presence of FLT3-ITD and FLT3-D835/ITD836 mutations. Whole genome sequencing of 799 pediatric AML samples from COG trials have shown novel FLT3 variants in patients where the tyrosine kinase domain also contains the 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.

**Aims:** Identify novel FLT3 mutations in pts with FLT3 mutant AML and further investigate whether these novel clones are sensitive to induction chemotherapy plus a potent pan-FLT3 inhibitor, crenolanib.

**Methods:** Pts with newly diagnosed FLT3 mutant AML were enrolled and treated with cytarabine/anthracycline/crenolani induction followed by high dose cytarabine (HiDAC) consolidation. Crenolani 100mg TID was started on day 9 of induction chemotherapy. Crenolani was stopped if variant FLT3 clones reduced by >60% on post-remission. Crenolani was continued for patients who relapsed on post-remission with the same FLT3 mutation, and who continued with crenolani. 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 Institution 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, A680V, and B411T/K (Table 1). The majority of these novel mutations were located at the JM, kinase domain 1 and the activation loop (kinase domain 2). The allele fractions of these FLT3 variants ranged as high as 29% (higher than that of FLT3-ITD in p33), 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 DNM3A mutations. All 4 pts achieved CR with full count recovery (3/4 pts achieved CR after just one cycle of cytarabine/anthracycline/crenolani induction). The pt with FLT3-D835Y and B411T/K achieved CR after cyparabine/anthracycline/crenolani induction and one cycle of HiDAC consolidation. All patients with FLT3-ve and have received FLT3-ve out of 4 pts received 1–4 cycles of HiDAC consolidation followed by crenolani maintenance. Only one pt underwent allo SCT. With a median follow up of 13 months, one pt relapsed (at 6.4-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.

**Table 1.**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age</th>
<th>FLT3 Mutation</th>
<th>Chemotherapy</th>
<th>Response</th>
<th>Median Follow-up (months)</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>V491L</td>
<td>CDA/CTHR</td>
<td>CR</td>
<td>12</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>V592L</td>
<td>CDA/CTHR</td>
<td>CR</td>
<td>18</td>
<td>CR</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>D593H</td>
<td>CDA/CTHR</td>
<td>CR</td>
<td>12</td>
<td>CR</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>A680V</td>
<td>CDA/CTHR</td>
<td>CR</td>
<td>12</td>
<td>CR</td>
</tr>
</tbody>
</table>

**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 crenolani in combination with standard induction chemotherapy has the ability to eradicate variant FLT3 clones. All 4 pts treated with chemotherapy followed by crenolani 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 and IDH1/IDH2 mutations have reported conflicting results. 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 FLRT3 (ITD or TKD), DNMT3A or NRRAS 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 is not affected by FLT3 mutations, or karyotypic abnormalities. 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 FLRT3-ITD, FLRT3-TKD or NRRAS mutations. For AML patients with IDH mutations who are eligible for induction chemotherapy “7+3” is a reasonable induction regimen regardless of the presence of FLT3 mutations, or karyotypic abnormalities.

Acute myeloid leukemia - Clinical 5

P554

VALIDATION OF PRECISION MEDICINE TEST FOR ACUTE MYELOID LEUKEMIA IN AN OBSERVATIONAL CLINICAL TRIAL

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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%).

haematologica | 2017; 102(s2) | 213
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 leveraging up to 45 different validated chemotherapy regimes. Assuming a similar response rate for all these treatments, our test could estimate a net prediction for sensitivity to AML treatment higher than 50% 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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1Department of Hematology and Oncology, University Hospital of Leipzig, 2Department of Hematology and Oncology, University of Leipzig, Leipzig, 3Department of Hematology and Oncology, Klinikum Chemnitz, Chemnitz, 4Department of Hematology, Oncology and Palliative Care, Klinikum Ernst von Bergmann, Potsdam, 5Department of Hematology, Oncology and Palliative Care, Klinikum Magdeburg, Magdeburg, 6Department of Hematology and Oncology, University Hospital of Halle, Halle (Saale), 7Department of Hematology and Oncology, Helios Klinikum Erfurt, Erfurt, 8Clinical Trial Centre Leipzig, University of Leipzig, Leipzig, Germany

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 followed 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 245%) and CR/CRi on d56 which were both previously identified as early predictors for long-term response to AZA in AML (Al-Ali et al. Leuk Lymph 2018). The primary endpoint was response (CR/CRi, and PR) at d90 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 achieved in 1stline AZA. Only lower baseline blasts correlated with blasts <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 (p=0.003). Of 152 AML cycles given till d36, 33.6% were applied in an outpatient setting. Until d90, one IC cycle was needed in 77 (65% of the intention) OR and mortality at d90 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/FIT3wt (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: Sequential response-based epigenetic and chemotherapy in elderly pts with AML is safe, associated with low mortality, and yields non-inferior responses compared to repeated cycles of IC. Marrow blasts d15 after the first AZA cycle and genetics could guide treatment-decision. The follow-up of this trial will scrutinize the impact of this approach on survival.

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 1). Additionally, a greater proportion of tAML patients in the CPX-351 arm versus the 7+3 arm achieved CR+CRi (47% vs 36%, 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=0/32 [0%]). Three (10%) patients in the CPX-351 arm and 5 (16%) 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 ferritinemia 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 promyelocytic 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 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 and 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 in 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 performing NGS analysis 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 centres (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 years (n=85), vs ≥75 years (n=54) (66.0 vs 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 yrs vs ≥75 years (10.2 vs 8.6 mutated genes/pt; P=0.030 and 12.9 vs 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 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>
<thead>
<tr>
<th>AML pts treated with azacitidine (AZA)</th>
<th>≤75 years</th>
<th>≥75 years</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n in pts.</td>
<td>85</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Mutated genes per median [range] (avg)</td>
<td>10.2 (9.0-13)</td>
<td>8.6 (7.0-11)</td>
<td>0.030</td>
</tr>
<tr>
<td>Mutations per median [range] (avg)</td>
<td>12.9 (10.4-23)</td>
<td>10.5 (8.0-18)</td>
<td>0.012</td>
</tr>
<tr>
<td>Results:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</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 & Vasiliou, 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 yrs vs ≥75 years (10.2 vs 8.6 mutated genes/pt; P=0.030 and 12.9 vs 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 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).
AML showed higher therapy-related mortality (TRM) rate. However, multivariate analysis showed that there were no significant differences between the three AML subgroups especially when the patients were treated with hematopoietic cell transplantation (HCT).

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 significantly shorter median OS of 33.5 days (P = 0.004; 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.

P562

INITIAL RESULTS FROM A FIRST-IN-HUMAN STUDY OF IMGN779, A CD33-TARGETING ANTIBODY-DRUG CONJUGATE (ADC) WITH NOVEL DNA ALKYLYATING 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, ImmunoGen, Inc, Waltham, 5University 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 in AML. IMGN779 comprises a humanized anti-CD33 antibody attached via a novel linker to the novel DNA-alkylating payload DGN462. Once released within the target cell, DGN462 exerts potent antitumor activity via DNA alkylation, without cross-linking, resulting in cell cycle arrest and apoptosis.

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 follows 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 sever-
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. In the highest cohort examined thus far (Cohort 5, 0.26mg/kg), sustained exposure (AUC) was observed in all patients through 48 hours post-infusion. A pharmacodynamics (PD) assay demonstrated consistent saturation of residual free antibody (AUC) was observed in all Cohort 5 patients past 48 hours, consistent with the PK results. Initial response data will be presented.

**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.

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**P563**

**COMBINATION OF TGR-1202, UBLITUXIMAB, AND BENDAMUSTINE IS SAFE AND HIGHLY ACTIVE IN PATIENTS WITH ADVANCED DLBCL AND FOLLICULAR LYMPHOMA**


1University of Nebraska Medical Center, Omaha, NE, 2Clearview Cancer Institute, Huntsville, AL, 3City of Hope National Medical Center, Duarte, CA, 4TG Therapeutics, Inc., New York, NY, United States

**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’s 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 (39%; 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.**

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<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
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<td>DLBCL</td>
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**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.

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**P564**

**VENETOCLAX (VEN) IN PATIENTS WITH RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA (NHL)**


1Dana-Farber Cancer Institute, Boston, United States, 2Royal Melbourne Hospital, Parkville VIC, 3Peter MacCallum Cancer Centre, East Melbourne VIC, 4University of Texas MD Anderson Cancer Center, Houston, 5University of Arizona Cancer Center, Tucson, Australia, 6AbbVie Inc., North Chicago, 7Memorial Sloan-Kettering Cancer Center, New York City, United States

**Background:** Venetoclax (VEN) is an oral BCL-2 inhibitor indicated for the treatment of patients (pts) with rel/ref diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL). VEN is FDA approved for DLBCL in combination with rituximab, with an ORR of 71% and a 22% complete response rate (Cheson, 2018). VEN exhibits 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.

**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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**Summary/Conclusions:** The combination of UTX, TGR-1202, and bendamustine has exhibited manageable activity 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), unacceptable toxicity, in dose cohorts ranging from 300 to 1200 mg. 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=3) or Waldenström macroglobulinemia (WM, n=4). Most common unacceptable grade treatment-emergent AEs were nausea (51%), diarhoea (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 (2-48 months) and on time on prior tx was 0.5 mo (0.2-1.2 mo). ORR was 75%, 6 pts (21%) achieved CR and remain on study (DORs: 25-40 mo). One pt with a PR proceeded to elective allogenetic stem cell transplant and remained disease free at last protocol defined follow-up (24 mo after coming off study). Median PFS was 11 mo and DOR was 15 mo. MZL pts (median age: 63 years) had received a median of 4 (2-6) prior tx. Time from start of prior tx to start of VEN was 8, 14, 73 mo and time on VEN was 5, 1, 35 mo. One pt (6 pt prior tx) received VEN for <1 mo due to progressive cytopenias; 1 pt (4 prior tx) achieved a PR with VEN at wk 6 but had PD at wk 16; 1 pt (2 prior tx) achieved PR at wk 6 and is the only pt to remain on study (DOR:32 mo). WM pts (median age: 67 years) had a median of 4 (3-5) prior tx. Time from start of prior tx to start of VEN was 5, 18, 33, 67 mo 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: VEN monotherapy has a tolerable safety profile in MCL, MZL and WM pts. ORR were high and most responses durable; median PFS and DOR suggest significant activity in MCL pts. Further investigation of VEN in each disease is indicated.

P565

WHOLE BODY DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING IS A GOOD PROGNOSTIC FACTOR IN TREATMENT OUTCOME AFTER ONE CYCLE OF IMMUNOCHEMOTHERAPY IN AGGRESSIVE LYMPHOMA

K. De Paepe1, F. De Keyser1, C.-A. Van Keerberghen2, O. Gheysens2, V. Vandecaveye1

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Background: Early identification of non-Hodgkin lymphoma patients not responding to therapy may enable treatment adaptation which might impact on the long-term survival of patients. Whole-body diffusion-weighted magnetic resonance imaging (DWI) has the potential to provide information about the tissue diffusion of water molecules, which can serve as a surrogate marker for tumour cellularity and therefore disease aggressiveness.

Aims: To evaluate the use of whole body diffusion-weighted magnetic resonance imaging (WB-DWI/MRI) as a radiation-free imaging technique to predict treatment outcome in NHL after one cycle of ICT (2-3 weeks).

Methods: Forty-six patients with aggressive NHL (35 diffuse large B-cell lymphoma (DLCLB), 2 primary mediastinal B-cell lymphoma (BCL), 1 unclassifiable BCL, 1 Burkitt lymphoma, 4 Mantle cell lymphoma (MCL), 2 peripheral T-cell lymphoma (TCL) and 1 extranodal natural-killer TCL) were consecutively enrolled between 2011 and 2015. All patients had baseline and interim WB-DWI/MRI after one cycle of immunotherapy, and end-of-treatment FDG-PET/CT. Aims: To further characterize the efficacy of VEN in patients with RR DLBCL, Richter’s transformation (RT) or FL.

Methods: 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, IBR dose and duration, reasons for discontinuation, and response. PFS and OS were estimated using the Kaplan Meier method and survival analysis by the log rank (LR) test.

Results: 44 patients were identified (DLBCL: 24, 54.5%; FL: 12, 27%, RT: n=8, 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).

Table 1.

Summary/Conclusions: 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 >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.

P567

PREVALENCE AND PROGNOSTIC VALUE OF MYD88 AND CD79B MUTATIONS IN IMMUNE-PRIVILEGED SITE AND (EXTRA)NODAL DLBCLS

J. Vermaat1, A. Amir2, M. Mindermann2, W. Kraan2,3, I. Saes1, L. de Wrede5, R. de Groen1, E. Kerver6, H. Berenschot1, W. Deelen7, J. Wegman9

1Lankenau Medical Center, 2Abramson Cancer Center, Hospital of the University of Pennsylvania, 3Hospital of the University of Pennsylvania, Philadelphia, United States

Background: Ibrutinib (IBR), a Bruton’s Tyrosine Kinase (BTK) inhibitor, is FDA approved for chronic lymphocytic leukemia, Waldenström macroglobulinemia, marginal zone lymphoma and mantle cell lymphoma. Despite its limited data, IBR is increasingly being utilized as a treatment option for patients with relapsed/refractory (RR) diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).

Aims: To evaluate the use of whole body diffusion-weighted magnetic resonance imaging (WB-DWI/MRI) as a radiation-free imaging technique to predict treatment outcome in NHL after one cycle of ICT (2-3 weeks).

Methods: 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, IBR dose and duration, reasons for discontinuation, and response. PFS and OS were estimated using the Kaplan Meier method and survival analysis by the log rank (LR) test.

Results: 44 patients were identified (DLBCL: n=24, 54.5%; FL: n=12, 27%, RT: n=8, 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).

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and other extranodal localizations (12%). In patients harboring a MYD88 mutation, indicating that these tumors represent distinct DLBCL subgroups. In patients presenting at IP sites. These patients with MYD88 mutations display a relatively high prevalence of coexisting CD79B mutations. Interestingly, a recent study by Wilson et al. (Nat. Med. 2015), indicates that these patients are more sensitive to treatment with Bruton’s Kinase inhibitors. Our study highlights the importance of investigating the mutational status of MYD88 and CD79B in larger prospective clinical trials with molecularly targeted agents, particularly in DLBCL patients with IP localizations.

P568
HIV-INFECTED PATIENTS WITH RELAPSED NON-HODGKIN LYMPHOMA (NHL) OR HODGKIN LYMPHOMA (HL): RESULTS FROM THE GERMAN HIV-RELATED LYMPHOMA COHORT STUDY
P. Schommers1, M. Hentrich2,*, D. Gillor1, C. Wyen1, T. Wolf3, J.-C. Wasmuth4, J. Bogner5, C. Spinner6, S. Esser7, B. Jensen8, M. Müller9, A. Schleicher10, G. Fätkenheuer1, C. Hoffmann11
1University of Cologne, Cologne, 2Hematology/Oncology, Rotkreuzklinikum München GmbH, München, 3University of Frankfurt, Frankfurt, 4University of Bonn, Bonn, 5University of Munich, 6University of Essen, Essen, 7University of Düsseldorf, Düsseldorf, 8Vivantes Auguste-Viktoria Hospital, Berlin, 9CH Study Center, Hamburg, Germany

Background: The outcome of HIV-associated lymphoma has undergone significant improvement in recent years beginning with the widespread use of combination antiretroviral therapy (ART). However, among AIDS-related deaths, non-Hodgkin lymphoma (NHL) is the most frequent event. HIV-positive patients (pts) with relapsed NHL or Hodgkin lymphoma (HL) should be treated in a manner similar to immunocompetent pts.

Methods: This prospective multicenter cohort study includes adult HIV-1 infected pts with biopsy or cytologically proven HIV-related lymphoma diagnosed at 32 participating centers in Germany and Austria since January 2005. Data on HIV-infection and lymphoma characteristics, treatments and outcomes were recorded. Pts with indolent lymphomas and primary central nervous system lymphomas were excluded from the present analysis.

Results: Of 499 pts (463 males, 36 females) 394 had aggressive NHL and 105 HL. The median age at lymphoma diagnosis was 45.6 yrs (range, 22–74). 344 pts (69%) were diagnosed with advanced stage (III/IV) lymphoma and the median CD4-cell count was 271/μl (266/μl in NHL and 287/μl in HL). Of 311 pts, 311 of 499 pts (62%) achieved a documented CR, 235 (60%) with NHL and 76 (72%) with HL. After a median follow-up of 17 months for NHL and 30 months for HL pts, 31 of 235 NHL (13%) and 8 of 76 HL (11%) experienced a relapse. Incidence of relapse was 6.9/100 patient years (PY) within the 1st year after primary diagnosis and 1.3/100 PY thereafter (P=0.0062). Median time to relapse was 7.3 months in NHL and 18.0 months in HL. Relapses beyond 12 months occurred in 6 of 31 NHL cases (19%) and in all 8 HL cases (100%) (P=0.045). Median overall survival (OS) of all relapsed pts was 29.0 months (95% CI 14.1-44.2 months) after primary lymphoma diagnosis. In pts with HL, OS was not reached, whereas it was 15 months in pts with NHL (P=0.024). Regarding the entire cohort of 311 pts with a documented CR, the 2-year OS rate was 57% in pts with relapse as compared to 97% in those without (P<0.001). The majority of relapsed pts died of lymphoma (86%).

Summary/Conclusions: Relapses from CR are relatively rare in pts with HIV-associated NHL and HL. In pts with NHL the majority of relapses occur within the first year after primary diagnosis, whereas in HL most relapses occur beyond 12 months. Overall, pts with relapsed HIV-related NHL have a worse outcome than pts with relapsed HL.

Figure 1. 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 CD79B mutations were identified in 51 patients and 19 cases, respectively. Interestingly, there was hardly any overlap between the presence of translocations (BCL2, BCL6 and MYC) or EBV and that of MYD88 and/or CD79B mutations, indicating that these tumors represent distinct DLBCL subgroups. In accordance with previous studies, the incidence of MYD88 mutations was increased at IP sites (67%, Chi-square P=0.001), compared to nodal (13%) and other extranodal localizations (12%). In patients harboring a MYD88 mutation, we frequently found a coexisting CD79B mutation (N=141). Patients with a
Background: Nodal peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of neoplasms, which include PTCL not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), anaplastic large-cell lymphoma (ALCL), anaplastic lymphoma kinase positive (ALK+), and ALCL-ALK-. Clinical assessments before and after treatment are essential to predict survival in nodal PTCL. 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 nodal PTCL were consecutively enrolled from 11 hospitals in South Korea. Patients were eligible if they were histologically diagnosed with nodal PTCL 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 by Deauville score 1-5.

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 ALCL-ALK+. Thus, 326 patients were analyzed. The median age was 61 years (range, 18-86) and 209 (64%) were male. PTCL-NOS (N=172, 53%) was the most common subtype included, and AITL (N=111, 34%) and ALCL-ALK- (N=62, 19%) followed. Fourths of patients (N=242) had stage III/IV. Majority of patients received anthracycline-based therapy. Patients were categorized into low (N=42, 13%), low-intermediate (LI, N=108, 33%), high-intermediate (HI, N=136, 42%), and high (N=40, 12%) risk groups according to NCCN-IPI. Based on the Deauville criteria, post-treatment PET scan was scored as 1 (N=130, 40%), 2 (N=47, 14%), 3 (N=60, 18%), 4 (N=27, 8%), and 5 (N=62, 19%). Because the number of progression in Deauville score 3 (40/60, 67%) was significantly different from score 2 (21/47, 45%; P=0.023) and 4 (24/27, 89%; P=0.030), we stratified patients into 3 groups: Deauville score 1-2, 3, and 4-5. With a median follow-up of 54.7 months (IQR, 30.2-84.5), 5-year PFS rate was 35.7% (95% CI, 30.0-41.4) and OS rate was 47.1% (95% CI, 40.8-53.4%).

NCCN-IPI risk and post-treatment PET-CT scan were independently associated with PFS in the multivariate analysis (for LI NCCN-IPI, hazard ratio [HR] 1.615, 95% CI 1.080-2.431; for HI NCCN-IPI, HR 3.063, 95% CI 1.626-5.769); high NCCN-IPI 4.475, 95% CI 2.231-8.977; P<0.001; for post-treatment Deauville score 3, HR 1.895, 95% CI 1.281-2.801; score 4-5, HR 6.916, 95% CI 4.948-9.667; P<0.001). We stratified patients into 5 groups based on risk of progression: a low (low NCCN-IPI and Deauville score 1-2), INT-1 (low NCCN-IPI and Deauville score 3, or LI NCCN-IPI and Deauville score 1-2), INT-2 (HI NCCN-IPI and Deauville score 1-2), high (high NCCN-IPI and Deauville score 1-2, or LI to high NCCN-IPI and Deauville score 3), and very high (Deauville score 4-5). The risk model showed a strong association with PFS and OS (Figure 1).

Summary/Conclusions: This study proposes a new risk stratification model incorporating baseline NCCN-IPI in combination with post-treatment Deauville score on PET-CT scan in patients with newly diagnosed nodal PTCL.

Results: Median age at diagnosis was 25.5 years (range 16-38 years) and 8/16 patients were male. Median previous line-therapies were 2.5 (range 1-5). In the Intention-To-Treat population which includes 1 patients who died after the first crizotinib dose, 13/16 patients (81.25%, 95% CI 53-95%) achieved an OR and a complete response (CR) after 1 month of therapy, respectively. Median overall survival and progression free survival (PFS) were 7.53 months and 4.57 months respectively (fig 1a). Median time to progression was 50 days (range 47-137 days). OS and PFS for 3 years from treatment were 44%. In 16 patients, 7 patients were still on treatment and in CR (median treatment duration 44 months [range 15-72 months]). There was a significant difference in 3 years PFS between patients in whom CR was obtained after 4 weeks of crizotinib and those who didn’t (PFS at 3 years 87.5% vs 0%, p<0.001-fig 1c); patients with less than 2 previous lines of therapy showed a borderline better 3 years PFS (66% vs 33%, p=0.08-fig 1b). Crizotinib was well tolerated and there were no cumulative adverse events (AEs) over this long-term follow-up. The only G3 AEs reported were transient neutropenia and creatine-kinase elevation. The deep sequencing of 4 NPM-ALK in relapsed patients demonstrated the presence in 2/4 samples of ALK mutations G1269A and C1156Y, which were not present in samples before crizotinib treatment. The level of in vitro resistance of these mutations showed a high level of resistance to crizotinib (resistance index for C1156Y and G1269A: 9.59-15.4 respectively). The sensitivity in vitro of these mutations to ALK-inhibitors was also evaluated: all inhibitors, except alecibib for G1269A, were active with a therapeutic index (TI) >20 (fig 1d). TI values, as previously reported by Mologni L. et al (Oncotarget. 2015 Mar 20;6(8):5720-34), provide a view of the therapeutic impact of a mutation: the bigger the value, the more targetable is the mutation with the inhibitor.

Figure 1.

Figure 1.
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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Background: Tipifarnib is a potent and selective inhibitor of farnesyltransferase (FT). FT catalyzes post-translational attachment of farnesyl groups required for the bone marrow and lymphoid organs and for maintenance of HSCs and immune cell progenitors. TIP12 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 of 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), 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 ATIL (N=12). Enrolled pts are treated with tipifarnib 600mg administered 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.

Figure 1.

Results: At data cut-off (2/15/2017), 18 pts (2 ATIL, 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 ATIL; 1 PTCL-NOS) and 3 additional pts experienced stable disease >6 months. Tumor DNA from 16 pts was sequenced using NGS tools, and all patients achieved ≥ 3'UTR CXCL12 single nucleotide variation (SNV) was observed. Seven of 16 pts carried the rs2389695 variant while an additional patient carried a novel variant. The presence of 3'UTR SNVs was associated with low levels of CXCL12 gene expression and disease progression (Figure). While all pts deriving clinical benefit from tipifarnib carried reference (wild type) 3'UTR CXCL12 and had tumors that expressed high levels of mRNA for this chemokine. Testing of circulating CXCL12 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 ATIL histology, absence of 3'UTR CXCL12 SNV and high levels of CXCL12 gene expression.

P572
BAM CONDITIONING BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA: A RETROSPECTIVE STUDY ON BEHALF OF THE FRANCOPHONE SOCIETY OF BONE MARROW TRANSPLANTATION AND AUTOLOGOUS STEM CELL TRANSPLANTATION FRANCOPHONE SOCIETY (SFctr)
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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 Promise database. 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 (35.7-59.9). Tumor stage (ASCT) was 295 days 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 and platelet (<50 Gigal/L without transfusion) recovery was respectively 11 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 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, 10%) and toxicity (n=16, 13%). Median overall survival (OS) was not reached and progression-free survival was 71.5 months [47-79]. 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 unfavorable 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 MICRONRANOEME, 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 inositol phosphatidyl anchored 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 small vesicles released by 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-). Total RNASE and without Eculizumab) using Total ExosomeIsolation kit (ThermoFisher).mRNA 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). Proteinic 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-100-5p, 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 miRNA in platelets and that has been associated with its reactivity) in 5.0- 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 V-I region HG3 increased in 3.9-fold in the Eculizumab group vs without Eculizumab group, which could be related with the treatment. Eculizumab treatment increased their expression, particularly in the group with thrombosis. 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 Diacylglycerol decreased in 17.3, 17.6 and 19.4-fold, respectively in patients treated with Eculizumab. Interestingly, the Aminoethylphosphonic acid, 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 extraheamotological 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 any group and share features of both of them. These “overlap 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 genes responsible for PIDs.

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%)ON patients had a PID genes mutation was found in a total of 8/24 patients (30) with 5 patients belonging to 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 ELANEin 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 cases.

Table 1.

P576
TREATMENT WITH HORSE-DERIVED ANTI-THYMOCYTE GLOBULIN LEADS 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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References
Acquired aplastic anemia (AA) is a rare disease characterized by bone marrow failure that results in pancytopenia and hypoplastic bone marrow. Hematopoietic stem cell transplantation (SCT) in SAA patients.

**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+ T cell, CD4+CD8+ T cell, CD4+CD28+ Tcell, CD4+ memory T cell and CD4+ naïve T cells. Simultaneously, we determined which factors influence immune reconstitution and analyzed the effect of immune cell subsect 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 naïve 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-matched 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.

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**P578 DEVELOPMENT OF A SCREENING AND DIAGNOSTIC ALGORITHM FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA USING A MODIFIED DELPHI PANEL METHODOLOGY**

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**Background:** Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoietic stem cell disorder that manifests with hemolytic anemia due to uncontrolled complement activation, bone marrow failure, and thrombosis. Diagnosis is essential because PNH is a progressive disorder associated with substantial morbidity and mortality. The protean clinical manifestations of PNH complicate diagnosis, and subsequently the diagnosis is often delayed or missed. Although national diagnostic guidelines are available, international expert consensus on PNH screening and diagnosis is lacking.

**Aims:** An international panel of PNH experts was assembled to develop a clinically relevant, consensus-driven screening and diagnostic algorithm for PNH.

**Methods:** An expert advisory committee of 4 PNH experts from North America, Europe, and Japan was assembled. Using a modified Delphi methodology, consensus was gained on the symptoms and signs of PNH and the laboratory tests required for screening and confirmation of diagnosis. Globally representative Delphi panelists were identified through a double-blinded screening process and asked to complete 2 rounds of web-based questionnaires. The questionnaires were developed by the expert advisory committee and presented to the Delphi panel in a case-based format. In the first round, Delphi panelists were given 5 blinded case studies—each including details on clinical presen-
tation and past medical history—and were asked to provide their differential diagnosis and the tests they would order to establish the diagnosis in free-text format. To reduce bias, Delphi panelists were blinded to the fact that the study was focused on PNH. Responses mentioned by ≥50% of Delphi panelists in the first round were included in the second-round questionnaire. For each case in the second-round questionnaire, Delphi panelists were presented with a set of 12 clinical consensus statements regarding potential diagnoses and the need for specific tests/data from a multiple-choice list and asked to respond to their level of agreement on a 4-point Likert scale. Consensus in the second round was attained if ≥80% of Delphi panelists agreed on a given screening or diagnostic approach.

Results: 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. Clinicians were also in agreement for 36 of 38 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 1) 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.

P579
DIAMOND-BLACKFAN ANEMIA IN THE NETHERLANDS: AN OVERVIEW OF CLINICAL CHARACTERISTICS AND UNDERLYING MOLECULAR DEFECTS
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Background: Diamond-Blackfan anemia (DBA) is a rare genetic disorder, characterized by bone marrow failure (anemia), congenital anomalies and a predisposition for malignancies. DBA is characterized by a highly heterogeneous nature, both clinically and genetically. Most of our understanding of this disorder stems from molecular studies combined with extensive data-input from international patient registries.

Aims: The aim of our retrospective study was to create an overview of the pediatric DBA population in the Netherlands.

Methods: Forty-four patients (age 0-18yr) diagnosed with DBA from all Dutch pediatric hematology centers included in this study.

Results: Congenital malformations were present in 19/41 patients (46.3%), varying from craniofacial and cardiac defects to urogenital and developmental disorders. An underlying genetic defect was identified in 23 patients (56.1%), the majority of which were found in the RPS19 gene (n=10; 45%). No significant diversities in malformations, course of disease or response to treatment were observed when comparing patients with or without identified genetic defects. In agreement with previous reports, two patients harboring defects in RPL11 displayed a more severe phenotype, including craniofacial malformations, thumb abnormalities, and cardiac defects. In contrast, our patient with a mutation in RPL5 has no associated congenital abnormalities, while previous studies have reported a very high frequency (83%) of associated congenital defects. Furthermore we observed a relatively high number (12/23) of novel mutations in well-known DBA-genes, defined as novel variants. In addition, we have identified a novel 33 kbp deletion in RPL9, in a patient with multiple congenital abnormalities (craniofacial defects, cardiac defects, colitis) in addition to severe anemia. Thirty-four (34/44) patients were treated with glucocorticoids, of which in thirty-one (31/34) patients a complete response was observed (91.2%). However, in 29% discontinuation was prompted by high-dose steroid independence, side effects, a weaning response, or a combination of these factors. Five patients (12.2%) were successfully transplanted with hematopoietic stem cells from either matched sibling donors (n=3) or matched unrelated donors (n=2), including two cases after the age of 10 years. Eleven patients (26.8%) were treatment-independent, defined as acceptable hematopoiesis without any therapy. No malignancies were thus far reported.

Summary/Conclusions: In line with previous reports, the Dutch pediatric DBA population is both clinically and genetically heterogeneous, with RPS19 being the most frequently mutated gene. Interestingly, the majority of mutations in our cohort have not been described before, probably further underlining clinical heterogeneity. In addition, we have identified a novel deletion in RPL9, associated with a more severe phenotype, based on multiple associated congenital defects. While we created a comprehensive overview of the 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.

P580
NEXT GENERATION SEQUENCING IN BONE MARROW FAILURE SYNDROMES
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Background: Inherited bone marrow failure syndromes (IBMFSSs) 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 IBMFSSs.

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 sequence coverage: classified IBMFSS (CBMFS) for those with a clinical picture typical on 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 93.9%, 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 autologous bone marrow transplantation (BMT). In ~50% of patients (pts) with AA, a clone deficient in glycosylphosphatidylinositol (GPI)-linked antigens—a paroxysmal nocturnal hemoglobinuria (PNH) clone—can be detected (Young, Blood, 2011). In recent years, new 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 aerolysin (FLAER); monocytes may also be analyzed but are not always evaluated 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, ISLH 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 > age 16 treated with IST. A total of 35 pts were included (with IST), the tertiary referral centre for the Province of BC, between 11/09 and 10/15. All patients had central pathology review and metaplasia 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
to detect the presence of a PNH clone. Granulocytes, monocytes and erythrocytes were interrogated with multi-colour flow panels including CD59 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 was 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 (SAA) or non-severe (NSAA)] 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 NSAA, 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 class-
PNC 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 NSAA and showed a trend toward an inferior response rate to IST (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Severity of AA</th>
<th>PNH Clone</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p value</th>
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</table>

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 NSAA 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.

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 pancytopenia 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. A 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) treatment, compared to 35-53% in rabbit-ATG (rATG) treated pts, considering hATG as first-line therapy in AA pts.

Aims: As response rates vary according to the different studies and the source of ATG being used, our aim was to retrospectively evaluate response rates in pts with AA receiving IST at the Department of Hematology at the University Hospital of Essen between 1988 until 2015.

Methods: In this single-center, retrospective analysis, approved by the institutional ethics committee of the University Hospital of Essen, data of all pts receiving IST to ATG, according to criteria reported by Camitta et al. 1975, were evaluated in 67 pts with AA (52% (35/67) females; median age 48 years (range 17-89 years)) being treated or monitored at the Department of Hematology between 1988 until 2015. 73% of the pts (49/67) were treated with hATG (ATGAM® (44/49) and Lymphoglobulin®). ATGAM® was administered at a dose of 40mg per kilogram (kg) body weight (BW) per day for 4 days and rATG (Thymoglobulin®) at a dose of 3.5mg/kg BW per day for 5 days, respectively. Pts in both arms simultaneously received CsA (5mg/kg BW) and prednisone (day 1-29).

Results: Following six months after primary ATG therapy, a hematologic recovery was achieved in 66% of the pts (44/67). The hematologic response rate at 6 months was 75% (37/49) for hATG and 39% (7/18) for rATG (p<0.005). Irrespective of the source of ATG we observed no significant difference in respect to gender (females: 71% (25/35) vs males: 59% (19/32)) or in the presence of a PNH clone (GPI-deficient granulocytes (FLAER) 67% (14/21) vs 79% (19/24) in pts with no detectable PNH clones), whereas in pts ≥50 years (yrs) a statistically higher rate in hematologic recovery was observed (≤50 yrs: 84% (31/37) vs ≥50 yrs: 43% (13/30); p<0.001). In primary refractory pts (34% (23/67)) (52%) (12/23) in first-line treated hATG pts vs 48% (11/23) rATG treated pts) a second course with either hATG (3/9) or rATG (6/9) was initiated, achieving an overall hematologic recovery at 6 months in 3 pts (33% (1/3) hATG vs 33% (2/6) rATG treated pts). A disease relapse (median: 13 months after primary ATG therapy) was seen in 11 out of the 44 pts with primary hematologic recovery (25%) (82% (9/11) in first-line treated hATG pts vs two rATG treated pts). A salvage therapy with rATG was initiated in two pts, whereas in one other pt a second course with hATG was started. An overall response following relapse therapy was observed in 33% of the pts (1/3). Four refractory as well as relapsed pts were treated with eltrombopag respectively (final results are still awaited). A secondary HSCT (hematopoietic stem cell transplantation) was performed in 11 out of the 67 pts (16%), either being primary refractory or due to a disease relapse.

Summary/Conclusions: Our data are able to independently confirm the findings of previous studies concerning hematologic recovery rates in pts with acquired 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.
Chronic lymphocytic leukemia and related disorders - Biology 2

P583
NOTCH1 MUTATED CHRONIC LYMPHOCYTIC LEUKEMIA CELLS ARE CHARACTERIZED BY A MYC-RELATED OVEREXPRESSION OF NUCLEOPHOSMIN-1 AND RIBOSOME ASSOCIATED COMPONENTS

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Aims: To gain insight into the biological and clinical significance of the presence of CLL-like B-cell clones from MBLlo individuals persist at increased counts after seven years of follow-up

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Background: The presence of very low numbers of clonal B cells in peripheral blood (PB) of otherwise healthy individuals (low-count monoclonal B lymphocytic leukemia (MBLlo) is a common finding in the general population. However, the vast majority of clonal B cells from MBLlo subjects show a phenotype overlapping with CLL (chronic lymphocytic leukemia) cells, the former might represent either the normal counterpart of CLL or the earliest stages of the disease. Little information exists about both the clinical outcome of MBLlo subjects and the biological features of their B-cell clones over time.

Aims: To gain insight into the biological and clinical significance of the presence of CLL-like MBLlo clones, we re-evaluated the biological features of clonal B cells and the clinical outcome of MBLlo individuals after 7 years of follow-up.

Methods: The baseline study was conducted in 2008, when 80 out of 639 (12.5%) healthy individuals (>40y) were found to carry at least one PB CLL-like clone. At the same time, 112 MBLlo clones were selected for subsequent analysis.

Results: A total of 64 CLL-like MBLlo clones (median size: 0.44 cells/ul, range: 0.02-767 cells/ul) were detected in PB of the 49 subjects at recruitment (in 15 cases ≥2 B-cell clones were detected in the same subject). In all subjects, B-cell clones persisted at reevaluation, phenotypically identical vs baseline. Interestingly, we found a near-uniform over-expression of MYC, characterized by a variable expression of MYC-related genes involved in protein biosynthesis (NPM1, which are allegedly responsible for cell growth and/or proliferation advantages of NOTCH1-mut CLL).
MICROENVIRONMENT REGULATION OF PROGRAMMED DEATH-1 (PD1) RECEPTOR AND ITS LIGANDS PD1L AND PD2L IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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 that anchor Lamin B1. 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 germline centre lymphomas and chronic lymphocytic 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 B cells. RNAi-mediated reduction of Lamin B1 resulted in spontaneous SHM and the nuclear envelope involved in epigenetic chromatin regulation, is reduced in B cells. The impact of Lamin B1 expression was performed in the absence of Lamin B1 in SHM in vitro. For in vivo studies, OVA immunised mice were used to study Lamin B1 dynamics in de novo formed spleen germinal centres. From a translational perspective, paired tissue microarray samples of diagnostic and transformed follicular lymphoma were analysed using immunohistochemistry and image analysis. Finally, comprehensive statistical analysis of CLL8 cohort patients was performed to test whether nuclear lamina was involved in the pathogenesis of 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 of B-cells showed heavy and variable immunoglobulin domains were released from the Lamin B1 suppressive environment when SHM was induced in B cells. RNAi-mediated reduction of Lamin B1 was performed in cellular and in vivo expression of SHM as well as kappa-light chain aberrant surface expression. Finally, Lamin B1 expression level correlated with progression-free and overall survival in chronic lymphocytic leukemia, and was strongly involved in transformation of follicular lymphoma.

Summary/Conclusions: In summary, here we report that Lamin B1 is a negative epigenetic regulator of SHM in normal B-cells and a “mutational gatekeeper”, suppressing the aberrant mutations that drive lymphoma malignancy.
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 effects. We have shown that microenvironmental signals (e.g., IL-4) can increase BCR expression and signalling, and can partially reverse the effects of BCR-kinase inhibition. ibrutinib, a drug that is known to determine an impairment of microenvironment, can allow the development of novel drugs that overcome resistance to kinase inhibitors. Cerdulatinib (cerd) is an inhibitor of both Syk (pivotal to BCR signalling) and JAK1/3 (integral for IL-4 signalling). Inhibition of Syk has been shown to induce apoptosis of CLL samples resistant to ibrutinib. Cerd is currently in phase II clinical trials in patients with relapsed/refractory B cell malignancies including CLL.

Aims: To investigate the effect of IL-4 on the regulation of BCR signalling in CLL and how this is modified by cerdulatinib.

Methods: Eighteen primary CLL samples were treated with IL-4 +/- cerd (1µM) and expression of FOXP1, GAB1, PTEN22, SOCS1 and SOCS3 assessed by immunoblotting. The effect of cerd on apoptosis was assessed by flow cytometry and PI/Annexin V staining.

Results: 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 PTEN22 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) which utilise either JAK1 or JAK3 for activation of STAT proteins. IL-4, CD40L and BCR ligation signals to CLL cells in lymph nodes can promote resistance to therapies such as the BCL2-inhibitor venetoclax. We have shown that cerd can overcome IL-4/CD40L induced expression of pro-survival proteins MCL1 and BCLXL and that cerd in combination with venetoclax could induce apoptosis in a synergistic manner in the presence of IL-4/CD40L.

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.
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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 (ISRCTN12650454).

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, 3, 4, & 12 months. The phosphorylation of Syk pY348, Btk pY551, ERK1/2, Akt pS473 was assessed in 4 conditions at each time point: unstimulated +/- ibrutinib, and stimulated with IgM/IgD +/- ibrutinib. 1x10^6 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 (10μg/ml). The BD phosflow protocol was followed to lyse/fix/permeate the CLL cells. Antibodies to Btk pY551, Syk pY348, ERK1/2 pT204/pY204, Akt pS473 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 pS473 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 pS473. Combinations of Btk inhibitor with a Syk or PI3 kinase inhibitor may result in complete BCR blockade. Phosphorylation patterns may also act as an adjunct to ascertain the response to therapy.
Background: Chronic myeloid leukemia (CML) is a myeloproliferative disease which arises in a haemopoietic stem or multipotent progenitor cell with the t(9;22)(q34;q11) chromosomal translocation. Tyrosine kinase inhibitors (TKIs) have been 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 LSCs therefore remains a major obstacle to cure CML. Understanding the mechanisms that LSC employ to survive TKI treatment is necessary to design essential therapeutics 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 which are important for self-renewal, DNA damage response, cell cycle arrest 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 or 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 minichromosome 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+38−90+r+93−) compared to CD34+38−90+r+93− cells. Next, we investigated the ability of heliophycinum (HQ), 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 prove the potency of HQ and its synergistic action in combination with Imatinib. We also investigated the changes in all of cell cycle and DNA damage response genes at the transcript level in response to HQ 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.
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 clinical trial of patients treated with BCR-ABL+/- LSC 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 re-processed for 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: 53.7 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 ± 32.0 % 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. Correlation with clinical data (Hasford score (R²=0.07, p=0.01) or 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.

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. There are only few reports to date that use next-generation sequencing (NGS) to look for somatic mutations - other than those affecting kinase domain of BCR-ABL1 - at the time of diagnosis (Dx) which could have a prognostic/predictive value.

Aim: We analyzed the mutational 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 died and paired samples from Dx and BP were analyzed); second group (MMR) included Dx samples from 36 patients who achieved major molecular remission (MMR) and TKI within 6 months and remained in MMR for at least 48 months from Dx.

Methods: Targeted enrichment strategy using custom designed capture probes (SeqCap EZ, Roche NimbleGen) followed by NGS on Illumina platform was performed in large genomic databases and our internal database were filtered out and the subsequent analysis was focused on putative protein damaging variants, supported by variant effect prediction tools such as PolyPhen-2, 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 (>80x) of all genes. Number of rare variants was 26 (range 18-38) and 29 (range 23-32) for DxC and progression samples respectively. In BP samples we detected new and previously described mutations in selected genes, which are frequently mutated in myeloid malignancies, namely in RUNX1 (36%, 4/11), DNMT3A (27% 3/11) IDH1/IDH2 (18%, 2/11) and ASXL1 (18%, 2/11). In 5 patients (54%, 6/11) mutations in these genes (excluding IDH2, detected only in BP sample) were preexisting at the time of Dx. These results were compared to second, control group that comprised of diagnostic samples from 36 patients (median age at diagnosis 53y, range 23-75) who were optimal responders to TKI and remained in MMR for at least 48mo (median time in MMR: 73o, range 45-128). In MMR group, the median number of rare variants was lower than in BP group (13 in Dx samples, range 4-14). Additionally, in 2 patients (25%, 5%) frameshift mutation in ASXL1 (p.Gly643_Gly644fs) was detected, identical as in one of BP patients. Additionally, one patient harbored RUNX1 mutation (p.Arg201Gln) which was not detected in the BP group.

Summary/Conclusions: Our results provide new insights into the already complex genomic landscape of CML. We suggest that a significant number of patients with poor disease outcome may harbor preexisting mutations in DNMT3A, RUNX1 and IDH1. In contrast, mutations in ASXL1 may be present at Dx in patients who will remain in long-term remission.
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≥4) 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 of 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, how they do so is not yet understood. The reason could be that high IDO activity may reflect endogenous IFN-α production, a known factor favoring immune-mediated CML-control. The predictive potential of KYN/TRP is currently verified in an independent cohort.

P596
BCR-ABL1 COMPOUND MUTANTS DISPLAY DIFFERENTIAL AND DOSE-DEPENDENT RESPONSES TO PONATINIB
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Background: Despite the dramatic improvement of prognosis in CML patients due to the introduction of tyrosine kinase inhibitors (TKIs), resistance to therapy 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. The IC50 values for this TKI exceed the maximum achievable effective plasma levels (eCFαave). 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 TKI therapy, therefore, to implement testing of the IC50 for ponatinib to prevent severe side effects should carefully consider the presence of CMs. The aim of our study was to assess the effect of BCR-ABL1 compound mutations on the sensitivity to ponatinib in vitro, we have introduced all BCR-ABL1 CMs into Ba/F3 cells using a recently developed multi-plate format. We report here the results of the study.

Methods: We have established a BCR-ABL1 protein model facilitating assessment of the presumptive impact of 27 different CMs involving important functional sites of the BCR-ABL1 TKD, and including constellations 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-based approach (Byrgazov et al., Oncotarget 2016, 7(47):78083-78094), and IC50 were derived from dose-response curves and plotted against the ECFαave values of the single mutations.

Results: Most CMs involving sites with no previous evidence for implication in resistance to ponatinib displayed IC50 values below 10 nM. This ECFαave 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 ECFα 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 be highly desirable, therefore, to implement testing of the IC50 for ponatinib and 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 adaptive 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 confounded with graft-versus-host disease (GVHD) and whether there is an allelogeneic 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 ascribe this therapy-free remission (TFR) a result of immune surveillance. Immune surveillance refers to a process whereby the innate and adaptive 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 confounded with graft-versus-host disease (GVHD) and whether there is an allelogeneic 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 ascribe this therapy-free remission (TFR) 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 worked with 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 (33 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 may 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 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.

P598 MUTATIONAL ANALYSIS IN BCR-ABL1 POSITIVE LEUKEMIA BY DEEP SEQUENCING BASED ON NANOPORE MINION TECHNOLOGY

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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 have 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, 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. MinION 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 A) and 12 (10 CML and 2 ALL cases) were observed in chronic phase (Group B) and no mutation was detectable by SS technique (33.4%) in MR5.0. On the other hand, of the 19 negative samples with the “LabNet” method, 2 (11.1%) were in MR4.0, 10 (55.5%) in MR4.5 and 6 (33.4%) in MR5.0. 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 worked with 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 (33 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 may 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 does not support the hypothesis that 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.

P599 THE AUTOMATED MOLECULAR TECHNIQUE “ULTRA” ALLOWS A SENSITIVE AND ACCURATE BCR-ABL1 QUANTIFICATION IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA

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Background: The chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia chromosome and the BCR-ABL1 fusion gene. The production of tyrosine kinase inhibitors (TKIs) significantly improved the survival, but 15% of patients don’t reach the optimal responses at the defined end-points or develop secondary resistance. The 2013 ELN guidelines identified as fundamental the early molecular response (BCR-ABL1/ABL1 % ≤10% IS), the MR3 (<0.1%) and the deep molecular response (MR4<0.01%, MR4.5<0.005%, and MR5<0.001%). Consequently, the molecular monitoring plays a crucial role in the clinical management of CML patients, with a consequent research of sensitive and standardized molecular techniques. The automated methods offer advantages in terms of reduced time for analysis, decreased manual steps, and reduction of possible errors and contamination. According to the American Society for Quality Control Technology (ASQCT) manual real-time PCR techniques standardized in the Italian network (57 centers), according to the European guidelines [Cross N, 2015]. We compared the sensitivity of the two methods (based on the number of ABL1 detected copies), the classification of molecular responses, with particular attention to the deep molecular subgroups.

Methods: We assessed the BCR-ABL1 transcript in 86 patients afferent to laboratories of Pisa, Napoli, Torino, and Bologna (Italy). For statistical analysis, the t-, the Pearson’s and the Cohen’s K test were adopted. Because our patients presented different transmural levels from the 10% to the 0% (MR4, MR4.5, MR5) the two techniques have been compared in the different molecular subgroups.

Results: Firstly we compared the number of detected ABL1 copies, that are fundamental for definition of the molecular response categories, especially for the main end points or develop secondary resistance. By the “LabNet” method, 51 (81%) samples exceeded the 100,000 copies of ABL1, while by the automated method 81 samples (94.2%) reached >100,000 ABL1 copies. Then, we compared the two methods in discriminating positive and negative samples (K Cohen=0.690; p<0.02). 77 samples were concordant (89.5%) and only 9 (10.4%) were discordant. Of the 18 negative samples with the “LabNet” method, 2 (11.1%) were in MR4.0, 10 (55.5%) in MR4.5 and 6 (33.4%) in MR5.0. On the other hand, of the 19 negative samples with the method “Ultra”, 1 (5.3%) was in MR4.5 and 18 (94.7%) in MR5.0, confirming the higher sensitivity of the automated method. In the cohort of positive cases by the two methods, the median values of transcript expression were superimposable (p=0.55) and the linear regression coefficient was very satisfying (Pearson’s r=0.9399; p-value <0.0001). Finally we compared the results produced by the two methods according to the “molecular classes” (MR1 vs MR2+MR3 vs MR4+MR4.5 vs MR5). This comparison showed a good concordance of the results in all the transmural levels in the study. In conclusion, our findings demonstrated high concordance between “Ultra” and “LabNet” methods using assay comparison criteria proposed by Müller et al. [Leukemia 2009] (Table 1).
Summary/Conclusions: In a huge 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

ROLE OF THE AURORA KINASE A/PLK1 AXIS INHIBITION IN RESTORATION OF CELL GROWTH CONTROL OF CHRONIC MYELOID LEUKEMIA PROGENITORS

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Background: Cell response to stress is a central component of genomic stability. The integrity of signaling pathways involved in cell cycle arrest, chromatin remodeling and DNA repair, are critical for the maintenance fidelity of replicated DNA. In this context, Gadd45 proteins function as stress sensors and de-transcription regulators. Gadd45α, in particular, intervenes in G2/M checkpoint induction and DNA repair, and it is required for efficient coordination of centrosome duplication hence preventing abnormal mitosis and aneuploidy. Such evidences let assume a putative role of Gadd45α in cancer development and progression, supporting the hypothesis that Gadd45α interacts with Aurora Kinase A (AKA), a key component of centrosome cycle and polar spindle assembly required for regulated progression from G2 to mitosis and throughout M. AKα is a member of a serine-threonine kinase family active during mitosis and it is frequently overexpressed in human cancers where correlates with a poor prognosis. Notably, AKα overexpression is always associated with defects in centrosome duplication, bipolar spindle and chromosomal segregation and aneuploidy, suggesting that it may enhance other oncogenic events by promoting genomic instability, one major trait of chronic myeloid leukemia (CML). Our aim is in the identification of a new molecular target able to control AKα activity. AKα cooperates with the constitutive activity of Bcr-Abl fusion protein by increasing DNA damage, promoting the occurrence of additional genomic alterations and driving TKIs resistance and disease progression to blast crisis.

Aims: Here we investigated AKα and Plk1 role in CML hematopoietic progenitor survival as potential targets to eradicate the transformed clone.

Methods: K562 cell line is a human cell line generated from a CML patient in blast crisis. Drug resistance was induced in K562 cell line by the exposure to progressively increasing doses of Imatinib (IM). It was validated by dose response curves showing a significant difference in LD50 of IM-sensitive and IM-resistant cells. By mean of cytofluorimetric and immunofluorescence microscopy analyses we investigated the events leading to AKα/Plk1 deregulation. Protein expression and activation were detected by western blotting and immunoprecipitation. Apoptotic cell death was measured by using an Annexin V/PI staining; cell cycle distribution was observed by PI staining and subsequent cytokinfluorenct analysis.

Results: Preliminary experiments were aimed to determine whether IM resistance in a BCR-ABL1 cell context is associated with the over-expression and hyper-activation of AKα/PLK1 axis. In our in vivo model drug resistance was associated with increased expression and phosphorylation of AKα (Y282) and Plk1 (T210). 24h exposure to IM significantly reduced expression and phosphorylation of both proteins in parental K562, but not in IM-resistant K562, indicating that AKα and Plk1 activation is only partly dependent on BCR-ABL1 TK activity. Subsequent experiments showed that the inhibition of AKα and Plk1 is in response to specific inhibitors (Danusertib and Volasertib respectively) was associated with:

- significant increase of gadd45α expression levels;
- reduction of cell survival;
- G2/M checkpoint arrest.

The findings support the role of AKα/PLK1 inhibition in restoration of signals involved cell growth control and apoptosis.

Summary/Conclusions: The advantage of using AKα and Plk1 inhibitors in CML therapy may come from effects independent from TK activity of Bcr-Abl protein. We proved that the AKα and Plk1 inhibitors induce growth arrest and apoptosis in IM sensitive and resistant cell lines.

P601

DURABLE TREATMENT-FREE REMISSION (TFR) FOLLOWING FRONTLINE NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTFREEDOM 96-WK UPDATE


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Background: ENESTfreeness (NCT017184068) is evaluating the ability to stop nilotinib and to maintain TFR in pts with a sustained deep molecular response (MR) on frontline NIL. Previous results from ENESTfreeness showed that 51.6% of pts (98/190) who attempted TFR remained off treatment and in major MR (MMR; BCR-ABL ≤ 0.1% on the International Scale [IS]) at 48 wk.

Aims: To analyze updated TFR data and predictive factors for remaining in TFR in ENESTfreeness.

Methods: Eligible pts had CML-CP with b2a2 and/or b3a2 BCR-ABL1 transcripts, ≥2y of frontline NIL, and MR4.5 (BCR-ABL IS≤0.0032%) prior to enrollment. All pts provided informed consent. After enrollment, pts continued NIL for 48 wk, and then were reevaluated for TFR at 96 wk. To investigate potential predictors for remaining in TFR, pts 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, or died, relapsed, progressed to blast crisis, or discontinued for any reason.

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 wk 48 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, 15.5% - 52.4%]), respectively, remained in TFR at wk 49 (Sokal risk scores were missing for 50 pts). Among pts with low, intermediate, or high Sokal risk scores at diagnosis, 39/62 (62.9% [95% CI, 45.2% - 60.6%]) remained in TFR at wk 48 vs 8/20 (40.0% [95% CI, 19.1% - 63.9%]) who had ≥1 assessment between MR4.5 and MR4.5 during the consolidation phase. Overall, 88 pts who reinstated NIL due to loss of MMR, 87 (98.9%) regained MMR and the remaining pt left the study 7.1 wk after NIL reintroduction 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. No pts had cardiovascular AEs during the study. No pts had 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.
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 Europe Against Cancer (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 QuantStudio instrument (Life Technologies) and expressed in the International Scale (%IS) and ddPCR (median: 0.01% IS, range: 0.0002–124% 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 breakpoint 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 transcript. Thus in qPCR analyses using the EAC protocol this may, at least on 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 BCR-ABL1 EAC protocol, the underestimation of the e14a2 variant could potentially be a widespread issue. We are presently working on an optimized BCR-ABL1 qPCR protocol where the e14a2 underestimation is eliminated.

5-YR RESULTS FROM THE PIVOTAL PHASE 2 PONATINIB PACE TRIAL: EFFICACY, SAFETY AND LANDMARK ANALYSIS IN HEAVILY PRETREATED PATIENTS (PTS) WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML)


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Background: Ponatinib is an oral tyrosine kinase inhibitor (TKI) approved for pts with CP-CML or Philadelphia chromosome—positive acute lymphoblastic leukemia (Ph+ ALL) for whom no other TKI therapy is indicated, or for pts with T315I. The ponatinib PACE trial (NCT01207440) enrolled pts with CML or Ph+ ALL resistant/intolerant to dasatinib or nilotinib, or with T315I. Long-term results in these heavily pretreated pts provide value in informing treatment decisions.

Aims: To report 5-yr efficacy and safety, and the association of early landmark responses with survival at 5 yrs past landmark, at 4 yrs past landmark in heavily pretreated pts with CP-CML from PACE.

Methods: Ponatinib starting dose was 45mg/day. Dose reductions were instructed in Oct ’13 to manage risk of arterial occlusive events (AOEs) observed with longer follow-up. Outcome measures were: 5-yr efficacy (n=267) and safety (n=270); post-study landmark analysis (n=267) of the association of molecular responses (BCR-ABL<0.1% [major molecular response (MMR)], >0.1–1%, >1%–10% and >10%) and cytogenetic responses (major [McYR] and 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.

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); and median %Ph+, 100% (2.5–100%); ≤10% Ph+, 20 pts (7%); 60% of CP-CML pts received ≥3 prior TKIs. In efficacy-evaluable CP-CML pts only, 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 15mg/d as their last ponatinib dose. In efficacy-evaluable CP-CML pts, cumulative response rates as of the data cutoff were: McYR, 60%; McYR, 54%; McYR, 40%; and McYR, 24%. Among pts who achieved McYR (n=148) or MMR (n=108), the Kaplan-Meier (KM) estimated probability of remaining in response at 5 yrs was 74% (95% CI, 62–83) and 61% (95% CI, 51–70), respectively. Maintenance of response was high regardless of dose reductions in Oct ’13. KM estimated 5-yr rates for PFS/OS were 49%–77%. Among pts with 3-, 6- and 12-mos landmark analyses, McYR, 54%; McYR, 40%; McYR, 39%; 62%/52% and 71%/56%, respectively, and MMR achieved in 14, 29% and 39%, respectively. Achievement of cytogenetic response and deep reductions in BCR-ABL1 levels (Table) at most landmark time points was associated with improved PFS and OS 4 yrs past landmark. Deeper responses at all landmarks were associated with achievement of McYR and overall survival with reduced adverse events (AEs) in ≥45% of CP-CML pts were rash 47%, abdominal pain 46%, and thrombocytopenia 46%. Most newly occurring AEs were observed within the first yr. The incidence of any AOE/serious AOE for CP-CML pts was...
Background: Very elderly (>75 yrs) people are a substantial proportion of elderly patients with newly diagnosed CML in chronic phase.

Aims: The aim of the study was to assess the treatment response and outcome in a cohort of very elderly patients with newly diagnosed CML in chronic phase.

Methods: We evaluated 435 patients ≥75 years of age who started imatinib 400 mg daily as first-line treatment. The estimated median age at diagnosis was 78 (31-86) years. The median follow-up from study entry was 141 months (range: 1-220 months).

Results: In univariate analysis, Hb level at diagnosis (≥ 12 mmol/l, p=0.048) was a significant predictive factor for achieving ≥MMR. Among patients ≥75 years of age, 71% (95% CI 66-76) achieved ≥MMR, compared to 89% (95% CI 87-91) of patients <75 years of age (p=0.0006). Male gender (p=0.046) and female gender (p<0.001) were significant predictive factors for MMR or better achievement.

Conclusion: As in younger subjects, nilotinib is effective in very elderly patients ≥75 years of age treated with nilotinib in chronic phase. Subsequent prospective studies are needed to validate these findings.

Summary/Conclusions: The long-term follow-up of very elderly CML patients treated with nilotinib suggests that any effort to treat these patients with standard doses should be made, in order to achieve cytogenetic and molecular responses as in younger subjects.
ease (including 7 acute myocardial infarction), 8 PAOD, 4 carotid stenosis (asymptomatic), 2 avascular necrosis of femoral heads, 1 optic artery ischemia, 1 aneurysm of carotid artery, 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 72% 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 in patients with or without ATEs (PPS: 96% vs 92%, p=0.55; 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

ASSASSEMENT 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 less than 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, 383 (65%) were male. Median age was 40 (17-84) years. 46 patients were received nilotinib (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 after 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 1.5-10.3, 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 DIGITAL-PCR (dPCR) IN CHRONIC MYELOID LEUKEMIA PATIENTS ACHIEVING MAJOR OR DEEP MOLECULAR RESPONSE WITH TYROSI-N-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 loose 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 (Q3SD 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 according 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 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

Figure 1.
discontinued TKIs, a preliminary analysis showed that 80% of patient with BCR-ABL1<0.48 copies/ul at discontinuation, maintained stable TFR (PPV of 80%).

Summary/Conclusions: This study suggests that dPCR is more precise and sensitive than qPCR when detecting levels of BCR-ABL1 transcript and that dPCR seems to be more robust and accurate for CML patients stratification.

Larger and prospective studies are warranted to confirm the higher sensitivity and accuracy of dPCR and its usefulness to better select the candidates for TFR.

P608

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 de novo CML-BP cohort OS of 11.03 mo (p=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% (6/23) 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 (35.7% vs 6.25%, p=0.02).

Summary/Conclusions: Our 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.

P609

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 (Garcia-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/ABL1 (IS) measurements were centralized in a EUTOS laboratory. Analyte to point were considered as non responders, Lympocyte counts, subpopulations and migration studies were done at baseline (1st day of dasatinib), and every 3 months, and they were done both previous to the dose, and 2 hours after.

Results: From April 2013 to May 2015, 18 patients were enrolled in 12 centers. Median age was 59 years (38-77), 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 (1.6-23) and median time while on imatinib to achieve MMR at 6 and 12 months. Interest 9/18 patients (50%) achieved MMR 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 number 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</th>
<th>Baseline</th>
<th>CML Baseline</th>
<th>CML4 Baseline</th>
<th>NK Baseline</th>
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<tbody>
<tr>
<td>Count (x 10³/µl)</td>
<td>7.8 (0.8-22.4)</td>
<td>9.0 (0.1-1.4)</td>
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<td>0.20 (0.02-0.87)</td>
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<tr>
<td>Percentage</td>
<td>27.0 (4.5-53.2)</td>
<td>28.2 (9.2-80.3)</td>
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Table 2.

<table>
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<th>No MMR 3m</th>
<th>p</th>
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<tbody>
<tr>
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<tr>
<td>CML Baseline (%)</td>
<td>36.4</td>
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<td>CML4 Baseline (x 10³/µl)</td>
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<td>NK Baseline (x 10³/µl)</td>
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</tbody>
</table>
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 obtainment of MMR at 3 months, a finding which underscores 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.

P610
GENETIC PREDICTION OF INSULIN RESISTANCE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH NILOTINIB
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Background: Impaired fasting glucose (IFG) and type 2 diabetes (T2D) represents adverse events in Chronic Myeloid Leukemia (CML) patients treated with the second-generation tyrosine kinase inhibitor (TKI) nilotinib. A genetic risk score (uGRS) for the prediction of insulin resistance, consisting of 10 multiple single-nucleotide polymorphisms (SNPs), has been proposed.

Aims: We evaluated the uGRS predictivity in 45 CML patients treated with nilotinib.

Methods: Patients were genotyped for IRS1, GRB14, ARL15, PPARG, PEPD, ANKRD55/MAP3K1, PDGFC, LYPLAL1, RSPO3, and FAM13A1 genes. The uGRS was based on the sum of the risk alleles within the set of selected SNPs.

Results: MR3.0 and CMR were achieved in 91% and 84% of the patients, respectively. Before treatment, none of the patients had abnormal blood glucose. During treatment and subsequently follow-up of 84.4 months (range 1-298), 5 patients (11%) developed diabetes requiring oral treatment, after a median of 11 months (range 3-95) since nilotinib. Nine patients (20%) developed prediabetes. Prediabetes/diabetes-free survival was significantly higher in patients with an uGRS below 10 compared to higher scores (100% vs 81%, p=0.004) (Figure). Each increment of 1 unit on the uGRS caused a 42% increase in the prediabetes/diabetes risk (HR=1.42; CI: 1.04-1.94; p=0.026).

Figure 1.

Summary/Conclusions: Although nilotinib is not associated with a higher incidence of T2D compared to a general population, it could be an early ‘‘heightener’’ of genetic predisposition to the disorder. The presence of more than 10 allelic variants associated to insulin secretion, processing, sensitivity and clearance is predictive of prediabetes/diabetes developing. In clinical practice uGRS could help tailor the best TKI therapy.

P611
THE EUROPE AGAINST CANCER PROTOCOL FOR BCR-ABL P210 TRANSCRIPT MEASUREMENT MAY OVERESTIMATE RESULTS FOR E13A2 AND E14A2 TRANSSCRIPTS
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Background: The quantitative PCR of BCR-ABL transcript has been the most useful technique for monitoring therapy in CML patients for over a decade. The numerous standardization projects have been undertaken in order to harmonize the molecular response results in laboratories all over the world. However, our data suggest that using the most common protocol may lead to overestimation of e13a2 transcript.

Aims: The goal of the study was to verify the observation that e14a2 transcript amplifies less efficiently than e13a2. The secondary goal was to validate the modification of Europe Against Cancer (EAC) protocol developed in 2011 which corrects observed artifacts.

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 calculation, 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 2.2 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 SIDERABLASTIC ANEMIA
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Background: Congenital sideroblastic anemia (CSA) is a inherited hemolytic 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 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 decreased ALA may represent a novel therapeutic strategy for XLSA, there is no model system to explore its use as an important tool for clarifying the molecular etiology of XLSA and providing a platform for drug discovery.

Aims: We aimed 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 cells (HiDEP) harboring a deletion of the ALAS2 gene (Δint1/Y) in male mice led to anembryonic lethality, suggesting that this sequence is indispensable in the context of sex. As an alternate approach, we established a clonal line with HiDEP cells exhibiting a red color, the XLSA clone appeared pink/pale color, which were accompanied by the significantly decreased intracellular heme concentration. Despite no obvious change in the expression of GATA-1 protein in the XLSA clone, quantitative real-time–polymerase chain reaction (RT–PCR) analysis demonstrated that the chromatin occupancies of GATA-1 and its cofactor TAL1 were significantly abrogated by the deletion of the GATA binding motif at intron 1 enhancer of the ALAS2 gene. Quantitative real-time–polymerase chain reaction (RT–PCR) analysis demonstrated significant downregulation of ALAS2 as well as globin genes (HBA, HBG, and HBB) in the XLSA clone. Microarray analysis revealed >2-fold up-regulation of 619 and 274 genes caused by the 19-bp deletion, respectively. The downregulated gene ensemble included globins (HBZ, HBG, HBE, HBD, HBM, and HBQ) as well as genes involved in iron/heme metabolism (ALAS2, transferrin receptor, TFR, copper/iron oxidase, CPOX, and mitoferrin 1: MFRN1). GO analysis revealed significant enrichment of cellular iron homeostasis (p=0.018), regulation of transcription (p=0.0021), and innate immune responses.

Results: We first generated a founder female mouse lacking the 1 enhancer region of Alas2, including the GATA binding domain (Alas2Δint1/X). Whereas the heterozygous Alas2Δint1/Y mice were viable and did not show any anemic phenotype, hemizygous deletion (Alas2Δint1/X) in male mice led to anembryonic lethality, suggesting that this sequence is indispensable in the context of mice. As an alternate approach, we established a clonal line with HiDEP cells exhibiting a red color, the XLSA clone appeared pink/pale color, which were accompanied by the significantly decreased intracellular heme concentration. Despite no obvious change in the expression of GATA-1 protein in the XLSA clone, quantitative real-time–polymerase chain reaction (RT–PCR) analysis demonstrated that the chromatin occupancies of GATA-1 and its cofactor TAL1 were significantly abrogated by the deletion of the GATA binding motif at intron 1 enhancer of the ALAS2 gene. Quantitative real-time–polymerase chain reaction (RT–PCR) analysis demonstrated significant downregulation of ALAS2 as well as globin genes (HBA, HBG, and HBB) in the XLSA clone. Microarray analysis revealed >2-fold up-regulation of 619 and 274 genes caused by the 19-bp deletion, respectively. The downregulated gene ensemble included globins (HBZ, HBG, HBE, HBD, HBM, and HBQ) as well as genes involved in iron/heme metabolism (ALAS2, transferrin receptor, TFR, copper/iron oxidase, CPOX, and mitoferrin 1: MFRN1). GO analysis revealed significant enrichment of cellular iron homeostasis (p=0.018), regulation of transcription (p=0.0021), and innate immune responses.

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: RESULTS OF A PROSPECTIVE NORDIC TRIAL
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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 clinical 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. Fludarabine and rituximab combination therapy showed 70% response rate (20% complete responses) and very long response duration, but considerably toxic.

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 obtained into complete response (CR), partial response (PR), and 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, 0–18). Seventeen patients had received previous therapy. At baseline, median Hb level was 9.5g/dL (range, 4.5–14.6), bilirubin 45micromol/L, lactate dehydrogenase (LDH) 468 U/L, haptoglobin undetectable, IgM 4.1g/L (1.0-2.72), CA titer 2048 (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 median of 4.8g/dL in the responders and 7.1g/dL in non-responders. 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 time. CR was achieved in a median of 7 months, 6 months in those achieving PR and 3.9g/dL in those achieving CR. Neutropenia grade 3/4 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 therapy-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 ENZYMIC 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...
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 anaemia 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
- To identify new disease causing genes.

**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 PK-R mutations. 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 Lorroca) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 20 μM AG-348 (37°C) prior to test. 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 the 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 increase of 0.2-2.2) similar to control cells (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 of 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 combined 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 the underlying pathologies of PK deficiency.

**P616**

IDENTIFICATION OF NEW PATHOGENIC MUTATIONS IN PATIENTS WITH RED BLOOD CELL MEMBRANE DISORDERS USING NEXT-GENERATION SEQUENCING

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2Background: 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 these patients was not usually performed before next-generation sequencing (NGS). In our institution we performed an initial screen by Sanger sequencing several membrane related genes, considering that they all contain a high number of coding regions.

**Aims:**

- The aim of this study is to perform the molecular diagnosis of the patients included in the study as well as to identify new pathogenic mutations leading to RBC membrane disorders.

**Methods:**

- 116 patients from 74 unrelated families were studied with a next generation sequencing (NGS) based panel that contained genes already described as disease causing for RBC membrane disorders (ANK1, EPB41, EPB42, SLCA4A, SPTA1, SPTB, PIEZO1, KCNN4, RHAG) as well as for enzyme deficiencies (ADA, AK1, ALDOA, BPGM, CYB5A, G6PD, GCLC, GPI, GSR, GSS, HK1, NTS3CA, PFKM, PKG1, PKLR, TP1), hemoglobinopathies (HBA1, HBA2, HBB) and congenital diseritrhopoietic anemias (CDAN1, C15orf41, SEC23B, KLFL1, GATA1, KIF23).

**Results:**

- Finally, with the NGS panel results, the genetic diagnosis of 89% (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.

**Summary/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 90% of the patients and it would avoid misdiagnosis or wrong therapy that could lead to splenectomy in conditions of apparent membranopathy. Moreover, the 11% of undiagnosed patients will be analyzed through a second NGS gene panel including potential new genes leading to chronic haemolyse and/or sequenced by whole exome sequencing with the aim to identify new disease causing genes.
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=0.01), 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 1.4-7.9) for Hb<10, 1.9 (1.4-6.2) for Hb<8, 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 LDLH levels and inadequate reticulocytosis, advising strict clinical observation in these patients.

P617

HEME BINDS ANNEXIN-A5 DURING HEMOLYSIS AND PREVENTS ITS INTERACTION WITH INTRACLASSICAL MEMBRANE PHOSPHATIDYLKEROSINE DURING SICKLE CELL DISEASE

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Background: Intravascular hemolysis, such as in sickle cell disease (SCD), is characterized by damage to red blood cells and intracellular vesicles in plasma, along with inflammation and tissue injury. Stressed leukocytes, platelets, endothelial and red blood cells shed microparticles (MP) that bear externalized phosphatidylinerse (PS) at their surface and promote tissue injury. Conversely, intracellular annexin-A5 acts as an inhibitor of extracellular annexin-A5 at the surface of cells and MP. Annexin-A5 is thought 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 annexins, and annexin-A5 activity in particular, is blocked by extracellular 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, with human serum albumin and hemopexin in competitive surface resonance assays. 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 bound readily to annexin-A5 with relatively high affinity in vitro, as demonstrated using absorbance shift, autofluorescence quenching and plasma surface resonance assays, with human serum albumin and hemopexin in competitive surface resonance assays. This was followed by annexin-A5 aggregation, which also produced a significant red-shift in heme absorbance wavelengths, implying that a tight and direct molecular interaction was possible. Hemoglobin and heme also triggered annexin-A5 aggregation in vitro, producing high molecular weight and heat-resistant multimers, observed by western blot. Surface plasmon resonance studies revealed that annexin-A5 assumes several sites for heme binding, some with very low affinity, while others are estimated with a Kd in the 10-6m range, rather similar to that of albumin. Part of the heme bound to annexin-A5 remained in place, even in the subsequent addition of the high-affinity heme-scavenger hemopexin. 3D molecular docking rendering suggested that heme may bind to the heme-binding site of annexin-A5, thereby preventing further interactions with PS. Finally, heme completely prevented the binding of exogenous annexin-A5 to purified PS+ MP and plasma MP, as well as their subsequent detection by flow cytometry.

Summary/Conclusions: Together, our data suggest that PS-neutralizing annexin-A5 is inhibited by cell-free heme. This heme-mediated inhibition of annexin-A5 may display physiopathological relevance, contribute to the accumulation of PS+ MP in plasma during intravascular hemolysis, and more specifically of RBC MP during SCD which can participate to the degradation of the vascular function.

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, dehydrated nitric oxide bioavailability (resulting in impaired endothelial function), infection, thrombosis and excessive vasoconstriction. These events lead to progressive peripheral vascular occlusion and subsequent tissue necrosis, such that even minor lower-leg wounds can become persistent ulcers, with no tendency to heal after months of appropriate treatment. PEGylated-Carboxyhemoglobin 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 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 32mg/kg (8 mL) for 4 or 6 weeks, as a once weekly infusion for either 4 or 6 weeks. The study 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 based on the study center. Subjects in Cohort 1 received 4 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 underwent a 3-week Follow Up Visit. Throughout, safety and efficacy were assessed using the Short Form-12 v2 Health Survey (SF-12), laboratory assessments (hematology, chemistry, and urinalysis), vital signs, concomitant medications, and 12-lead electrocardiograms (ECGs), efficacy: wound pain, wound appearance and condition, wound size, wound vascular status (Venous Clinical Severity Score; VSCC), quality of Life: Quality of life was assessed using the Short Form-12 v2 Health Survey (SF-12).

Results: The administration of once-weekly infusions of PEG-COHb was well tolerated. Treatment emergent adverse events (mild pyrexia, moderate wors-
ening anemia) considered related to study drug were reported in 2/10 patients. Increases in hepatic enzymes were anticipated due to the chelatic effects of this coloidal 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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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 (hemoglobin level, reduction status, hemoglobin ratio, alpha globin status, inflammation, serum ferritin and renal function).

Methods: King’s College Hospital (London, UK) has a large SCD population. All patients with HbSS or HbSβthalassemia who had a serum EPO level measured between 2007 and 2013 were included. Sickle genotype, alpha genotype, “baseline” HbF (no hydroxycarbamide, transfusion or pregnancy) and demographic data were recorded. Other clinically relevant variables were obtained from the same day as EPO levels (medications, laboratory values and oxygen saturation). Serum EPO was measured by chemiluminescence immunoassay (Siemens Immulite XPi). Exclusion criteria were: active vaso-occlusive crisis, transfusion within 8 weeks, chelation, erythropoiesis stimulating agent therapy, 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: 245 adult (≥17 years) SCD patients (all of African or African-Caribbean origin) met the inclusion criteria. Of the 245, 241 had HbSS and 4 HbSβ0, 100 were male and 145 female, and mean age was 31.2 +/- 11.6 years. 221 had genotype, “baseline” HbF (no hydroxycarbamide, transfusion or pregnancy) and demographic data were recorded. Other clinically relevant variables were obtained from the same day as EPO levels (medications, laboratory values and oxygen saturation). Serum EPO was measured by chemiluminescence immunoassay (Siemens Immulite XPi). Exclusion criteria were: active vaso-occlusive crisis, transfusion within 8 weeks, chelation, erythropoiesis stimulating agent therapy, 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.

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Results: 245 adult (≥17 years) SCD patients (all of African or African-Caribbean origin) met the inclusion criteria. Of the 245, 241 had HbSS and 4 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 52.8 kg (range 44.6 to 65.8kg). GBT440 was well tolerated; there were no drug-related 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.

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<th>Parameter Estimate</th>
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<tr>
<td>T1/2 (h)</td>
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P620
THE PHARMACOKINETICS (PK) OF GBT440 ARE SIMILAR IN ADOLESCENTS AND ADULTS WITH SICKLE CELL DISEASE (SCD)
C. Washington1, R. Savić2, A. Inati3, J. Estepp4, G. Woods5, E. Fong1, A. Hutchilelela1, M. Tonda1, G. Balaratnam1, J. Lehrer-Graiwer1
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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 HbS βthalassemia). 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 52.8 kg (range 44.6 to 65.8kg). GBT440 was well tolerated; there were no drug-related 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.

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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.
Gene therapy, cellular immunotherapy and vaccination

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 proliferative and is also a biologic antigen for CD8+ 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-A*24 ATL patients who underwent allogeneic stem cell transplantation (allo-SCT). We found that a particular amino acid, tyrosine (Tyr), in the T-cell receptor (TCR) (CDR3 region of TCR-ß) was conserved in different patients and also within the same patient before and after allo-SCT, and the PDR Tyr+ T-cell 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-tetramer 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 efficency 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-ß genes were cloned from an established PDR+ clone and integrated into a retroviral CR vector (Tax-siCTLs). First, cytotoxicity and cytokine production capability of the Tax-siCTLs and ATL-cells were evaluated using calcine-am-fluorescence assay and flow-cytometry analysis, respectively. Next, to evaluate the in vivo anti-ATL effects by the Tax-siCTLs, the bioluminescence assay (in vivo imaging system) was performed. We generated a luciferase-transduced HLA-A*24-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-2RKO Jic (NSG) mice intraperitoneally. After 3 weeks, 2 × 106Tax-siCTLs were administered, in comparison, non-integrated T-cells (Mock) were administered in the same way. These mice were monitored for tumor growth using IVIS system weekly.

Results: Tax-siCTLs showed specific and strong killing activity against both HTLV-1 infected T-cells and patients' ATL-cells without any reaction against normal cells. In addition, Tax-siCTLs produced a sufficient amount of cytokines such as IFN-γ, TNF-α, and IL-2 against HTLV-1 infected T-cells. In mice experiments, the bioluminescence of Luc-MT-2 in the mice treated with Tax-siCTLs 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 treated mice with Mock or in the control mice without treatment had rapidly increased and all mice died by 9 weeks.

Summary/Conclusions: We confirmed that Tax-siCTLs 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-siCTLs has a potential to be a novel immunotherapy for ATL patients.

P624
NOVEL ENHANCED AND DUAL TARGETING CAR INVARIANT NK CELL-BASED IMMUNOTHERAPY FOR CD1D+ B CELL MALIGNANCIES

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Background: Anti-CD19 chimeric antigen receptor T cell (CART19) immunotherapy has shown promising clinical potential in relapsed/refractory mature B cell malignancies. However, only about half of patients benefit, highlighting the need for more effective CAR-based strategies. iNKT cells are rare but powerful immunoregulatory and cytotoxic T lymphocytes, playing a pivotal anti-tumor role. iNKT cells are restricted by CD1d, a non-polymorphic, phospho-glycolipid-presenting HLA I-like molecule. We previously showed that CD1d, as well as on normal B cells, is also expressed on malignant CD19+ B cells in mantle cell lymphoma (MCL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL).

Aims: We tested the hypothesis that bi-specific CAR/iNKT cells targeting simultaneously CD19 and CD1d via the CD19-specific CAR and their natural invariant TCR respectively, would be more effective than CART19 cells against CD19+CD1d+ B cell malignancies.

Methods: We optimized a novel protocol for manufacturing 2nd (CAR2) and 3rd (CAR3) iNKT cells expressing CAR19. Their in vitro reactivity was assessed in cytotoxicity (flow cytometry-based) and cytokine and cytotoxic granule release assays (intracellular staining and LumineX technology). In vivo reactivity was assessed in NSG xenograft assays, with monitoring of 1T1CD1d1 tumour cure for CART19 and bi-specific CAR/iNKT cells.

Results: Our optimised protocol for selection, lentiviral transduction and clinical scale expansion of CAR/iNKT cells within 3 weeks is suitable for frozen and fresh lymphocytes, derived from either healthy donors or cancer, including lymphoblastic cell lines (LCLs) and primary HSCPs were targeted by the CRISPR/Cas9 system. INDels generated in edited FA sequences in these cells.

Methods: Two different FA mutations from FA-A patient-derived lymphoblastic cell lines (LCLs) and primary HSCPs were targeted by the CRISPR/Cas9 system. INDels generated as a consequence of the NHEJ mechanisms were then analyzed at different time points.

Results: Initial studies conducted in a FA-LCL carrying the biallelic c.295C>T point mutation that generates a premature stop codon (p.Q99X) showed targetting efficiencies around 20%. Next Generation Sequencing (NGS) not only revealed the generation of frame-restoring repair events, but also that these NHEJ events have been converted to edited cells. Moreover reversion of the characteristic MMC hypersensitivity and restoration of the FANCD2 foci formation were observed in these cells. In addition, western-blot analysis confirmed the stable expression of FA protein. To further demonstrate the feasibility of the approach, a second FA-A LCL carrying the c.3580insG, producing a frameshift and a premature stop codon -p.R1187fsX28- with even higher gene targeting efficiencies. Finally similar studies were conducted in three HSCPs samples from FA-A patients harboring the c.295C>T mutation, that showed targetting efficiencies up to 36%. Moreover, NGS detected the presence of corrective NHEJ-repair events immediately after editing and evidencing up to 50-fold expansion of corrected cells after nine days in culture, confirming the functionality and proliferative advantage conferred by the frame restored alleles.

Summary/Conclusions: Our results demonstrate for the first time that NHEJ gene correction is feasible in FA-HSCPs. The high efficiency of the NHEJ repair pathway in HSCPs together with the simplicity of the strategy, may approach clinically relevant for the future treatment of the hematopoietic defects in FA patients.
phoma patients. The manufacturing process consistently allows high CAR transduction efficiency of NK and T cells (75.31%±4.294 and 76.95%±14.76 respectively, n=8) and ensures the preservation of CD4+ NK 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 CD1d-restricted reactivity and exert additive dual-specific cytotoxicity against CD19+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-NK cells are equally or more effective than their CART counterparts at killing CD19+ and CD19-Lewis cell lines (B-lymphoblastoid 1RCD1d and lymphoma-derived Farage 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 NKT cell-treated animals was the same as that of untreated animals (P=0.20), 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).

Summary/Conclusions: In our pre-clinical in vitro and in vivo lymphoma models, CARiNKT19 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 iNK 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 on 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 recombinant 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: The 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, HS62, Peer, Jurkat), B-cell ALL (OP-1), Burkitt’s lymphoma (Raji), osteosarcoma (NOS-10, NOS-1, NOS-2, SaOs-2, U2OS,mg-63), rhabdomyosarcoma (RMS-YM, Rh28), and neuroblastoma (NB1, NB16, IMR-32, SK-N-SH). Different expression levels of CAR 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 CD3ξ. A combination of the hinge domain from NKp44, transmembrane domain from CD8α and intracellular domain from CD3ξ 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 may be suggested to play a role in ligand binding (Koch J, 2013), but the details are poorly understood. Intriguingly, replacement of 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 activities 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 DIRECTED HUMAN T LYMPHOCYTES INDUCE POTENT ANTITUMOR IMMUNITY TO LEUKEMIA CELL LINES AND PATIENT-DERIVED ACUTE MYELOID LEUKEMIA IN NSG XENOGRAFT MODELS
P. Ploch1, S.A. Khan1, M. Theobald1, U. Hartwig1,*
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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 carrying T cells induce potent immune responses. We investigated the potential of the recombinant human NKp44 Fc chimeric protein.

Methods: We created several NKp44-based CAR constructs. Human T cells transduced with this CAR 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 carrying T cells induce potent immune responses. We investigated the potential of the recombinant human NKp44 Fc chimeric protein.

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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 under-
scores 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 potential of adoptively transferred antigen-specific T cells. We also proposed 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 (s.c.) with HLA-A2.1-expressing MDM2+/- p53+/- cell line. In the adoptive cell transfer approach, mice were treated (i.p) with anti-PD-1 (Nivolumab). Tumor growth was monitored and intratumoral alterations (in particular expression of relevant tumor and T cell antigens) in ex-vivo tumors were analyzed by flow cytometry. Tumor infiltrating lymphocytes (TILs) were also characterized by flow cytometry.

Results: Adoptive transfer of dual MDM2/p53-specific TCR equipped T cells showed a superior anti-tumor response in vivo compared to single TCR treat-
ment, 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 no significant infiltration of TILs in TILs samples which may limit the efficacy of antigen-specific TILs. Accordingly, in vivo ACT experiments combined with anti-PD-1 inhibitor, demonstrated the synergistic therapeutic potential of this approach as compared to single agent. Yet, it does not result in complete tumor eradica-
tion suggesting that targeting one single immune checkpoint receptor is not sufficient to drive a full anti-tumor response.

Summary/Conclusions: Combination checkpoint inhibitor approach has demonstrated promising potential in our ACT experimental MM model and forms the basis for a novel multi-modal immunotherapeutic combination treat-
ment for multiple myeloma.

P628
ENGINEERED T CELLS TOWARDS BAFF RECEPTOR: A NOVEL STRATEGY TO EFFICIENTLY TARGET B-CELL ACUTE LYMPHOLASTIC 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. Immunother-
apic approaches targeting the CD19 molecule paved the way for the treat-
ment 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 shown 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) approach and its immunotherapeutic potential aiming at re-directing adoptively transferred antigen-specific T cells.

Methods: We characterized the expression of BAFF-R in B-ALL primary sam-
ples. As immunotherapeutic approach to target BAFF-R molecule, we de-
veloped six anti-BAFF-R.CARs that differ for the inversion of the VH and VL and the length of the spacer domain. Cytokine-induced Killer (CIK) cells, an heterogeneous populations of CD3+ effector lymphocytes with acquired NK-like cytotoxicity enriched in highly effi-
cient cytotoxic CD3+CD56+ cells, were engineered using an improved Sleeping Beauty (SB) transposition platform and used as effector population.

Results: Adoptive transfer of BAFF-R specific TCR engineered in B-ALL primary samples at the onset and relapse. In order to develop a chimeric antigen receptor (CAR) approach targeting BAFF-R molecule, six anti-BAFF-R CAR genes 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) transposition system, stably expressed anti-BAFF-
FR.CARs, and maintained their characteristic phenotype. Among the newly con-
structed CARs, the shortest VHH anti-BAFFR CAR exerted the highest anti-
leukemic activity towards target cells, such as NALM-6, in an in vitro killing assay. These data supported the use of BAFF-R CARs developed in terms of cytokine release by intracellular staining (8,9±2.6% of IFN-γ and 16,4±5.5% of IL-2 pro-
ducing cells). Importantly, we also detected a specific cytotoxic activity towards primary B-ALL blasts (average 65,6±4,5%, n=9). Combining the INvSh.CAR with CD19.CAR we detected a superior antitumor activity towards ALL targets (average in vitro killing ability of 72,2±22,9% of NALM-6 and 87,1±13,2% of B-ALL blasts) compared to single population per se. 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 valid candidate target for CAR engineering 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 apia-
sia observed in patients treated with anti-CD19 CAR-modified T cells.
BACKGROUND: Acute Myeloid Leukemia (AML) is an aggressive malignancy associated with high relapse rates when treated with conventional chemotherapeutic and hematopoietic transplantation regimens. In search for alternative strategies, interest has focused on antigen-specific 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 the BB305 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 (≥100 mL/kg of packed red blood cells [RBCs] per year) were enrolled.

RESULTS: CD33.CAR-CIK cells were infected 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-CKI cells is assessed by means of cytoxicity, proliferation and cytokine production assays upon challenge with AML cell lines and primary samples. The in vivo efficacy of CD33.CAR CIK cells is evaluated in NSG mice transplanted with AML cell lines (MA0-NRas cells) and primary samples. Moreover, to investigate the potential benefit of CD33.CAR CIK cell immunotherapy in combination with standard-of-care treatments, xenograft chemotherapy models is exploited, by using standard AML induction therapy drugs (Ars-C and doxorubicin).

Summary/Conclusions: Having demonstrated the significant in vitro anti-CD33.CAR-CKI 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/multioid progenitor cells. Finally, if successful, our results will provide the preclinical validation of CD33.CAR-CKI 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-thalassaemia trait (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 β(0)β(0) genotypes and 1 is homozygous for a severe β(0) mutation (IVS1 nt 110 G>A). Two of the β(0)β(0) 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 HbA(T7Q) of 7.7 and 10.1 g/dl, respectively. The third patient with a β(0)β(0) 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 HbA(T7Q) 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 HbA(T7Q) of 6.7 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 Hb(T7Q) 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.
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 cytopenias, 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 dependence was noted in 20.3%, splenomegaly in 27.9% and bone marrow involvement in 69.2%. 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 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/L (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 INCB050465, A SELECTIVE PI3K-DELTA INHIBITOR, FOR THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY B-CELL MALIGNANCIES (CITADEL-101)
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Background: Signalizing networks mediated by PI3Ks have been implicated in proliferation, migration, and function of B-cells. INCBO50465 is a novel, potent, and selective PI3Kδ 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 IC50 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 8 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 [20–85], 50% were male). 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 ≥3 prior systemic regimens; 31% (n=16) had prior HSCT. Median duration of therapy was 4 months (range, 0–14); 15 patients were treated ≥6 months. Responses were assessed in 55 patients who received ≥2 dose levels of INCBO50465 (17%), lymphopenia (10%), and anemia (6%). Forty percent of patients had serious adverse events (AEs), most frequently colitis, diarrhea, and hypotension (24%); 10% of patients had grade 3 pneumonitis; none had Pneumocystis jirovecii pneumonia (PJP) or grade ≥4 elevated transaminase. Objective responses occurred at all doses (Table 1), except 5mg QD; 90% of the objective responses were observed in 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 transaminitis 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.

P635
PHASE III RANDOMIZED STUDY OF LENALIDOMIDE PLUS RITUXIMAB (R2) FOLLOWED BY LENALIDOMIDE VS. RITUXIMAB MAINTENANCE IN PATIENTS WITH RELAPSED/REFRACTORY NHL: ANALYSIS OF FOLLICULAR LYMPHOMA PATIENTS
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Background: Lenalidomide is an immunomodulatory agent with direct and immune-mediated mechanisms of action, and clinical activity in indolent non-Hodgkin lymphoma (NHL). Recent studies in frontline and relapsed/refractory (R/R) INHL show tolerability and high activity for the combination of lenalidomide plus rituximab (R2) and support further study of R2. Aim: The current study analyzed the efficacy and safety of R2 induction in patients with R/R follicular lymphoma (FL).

Methods: MAGNIFY (NCT01996865) is a Phase IIIb, multicenter, open-label study of R/R NHL patients, including grades 1-3b and transformed follicular
lymphoma (IFL). Upon informed consent, patients receive 12 cycles of R² induction (indomethacin 200mg, 3 weekly cycles 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 y (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 (87%) 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 of ≤6 mo after the last rituximab dose). The most common prior regimens were rituximab alone (40%), R-CHOPI-R-CHOP-like (38%), and bendamustine plus rituximab (35%). Preliminary discontinuation rates of R² induction occurred in 30 (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’ study 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 CVB 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) (NCT01262771).

Methods: A total of 140 patients were randomized in a 1:1 ratio to receive CT-P10 or RTX (375mg/m² intravenous) plus CVB (cyclophosphamide, vincristine, and prednisone) therapy every 3 weeks over 8 cycles. Overall response rate (ORR) was the primary endpoint and assessed by independent review committee, according to the 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).

Summary/Conclusions: This study demonstrates therapeutic non-inferiority of CT-P10 to RTX combined with CVB therapy in previously untreated AFL Patients well-tolerated and the safety profile including immunogenicity of CT-P10 was comparable to that of RTX over 8 cycles in induction period.

Table 1. Summary of Efficacy [Number (%) of patients].

Table 2. Summary of Treatment-emergent adverse event (TEAE) related to the study drug [Number (%) of patients].

Summary/Conclusions: This study demonstrates therapeutic non-inferiority of CT-P10 to RTX combined with CVB therapy in previously untreated AFL and the safety profile including immunogenicity of CT-P10 was comparable to that of RTX over 8 cycles in 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 low-dose IFN-α2b (6-18 MU weekly) and PUVA, which was first reported 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.

Results: Eighty-nine patients (56 men and 33 women) with a median age of 60 years (range, 17-80) were recruited. Disease stage IA was 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 (95% CI 79%-89%). 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 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 PVU. 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 PVU 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 regimens set the realistic goal of achieve high rates of complete clearance 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 PVU and IFN-α compared with either agent alone. With respect to Hughes et al. (Blood 2015), our combination treatment provides a longer TTNT than PVU or IFN-α monotherapy (36.3 months and 27.9 months, respectively). At 2 years, 95% of patients receiving PVU plus IFN-α were free from further treatment as compared to 54.2% and 29.1% treated with PVU or IFN-α monotherapy, respectively.

P637

PHASE 3 ALCANZA STUDY: THE BREVITUXIMAB VEDOTIN (BV) OR PHYSICIAN’S CHOICE (PC) OF METHOTREXATE (MTX) OR BEXAROTENE (BXE) 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 BXe) 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 (PFS; 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 additional event of disease progression or death compared with PC in the ALCANZA trial. To determine the NNT with BV to avoid one additional event of disease progression/death compared with PC in the ALCANZA trial.

Methods: The NNT with BV was calculated as the inverse of the absolute risk reduction (ARR); ARR was the PFS event rate per independent review facility (IRF) assessment in the PC arm minus the event rate in the BV arm. PFS was defined as the time from randomization until progressive disease/death due to any cause, counting all events despite two or more missed visits or starting of subsequent anticancer therapy (European Medicines Agency [EMA] criteria). ALCANZA recruited adults (≥18 years) with previously treated CD30+ mycosis fungoides or primary cutaneous anaplastic large cell lymphoma. Pts were randomized 1:1 to receive BV 1.8 mg/kg IV, once every 3 weeks, for up to 16 three-week cycles, or PC of MTX 15-50 mg PO, once weekly, or BXe 300 mg (target dose) PO, once daily, for up to 48 weeks. All pts gave informed consent. Results: The intent-to-treat (ITT) population comprised 128 pts (median age 60 yrs [range 22–83]; 55% male) who received BV (n=64) or PC (n=64). Fewer PFS events per IRF assessment per EMA criteria were experienced by pts in the BV arm (Table). The NNT was 9.6 (95% CI 7.5, 12.4) at a disease progression/death rate of 2.00 (95% CI 1.59, 2.36) to 3.76 pts (95% CI 2.5, 5.84) over 24 months (Table). At 24 months, the NNT to prevent a disease progression/death was 3.37 pts (95% CI 2.26, 7.67).

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>PFS (BV)</th>
<th>NNT</th>
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<tr>
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<td>24</td>
<td>6</td>
<td>1.43</td>
<td>0.78</td>
<td>2.17</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.

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/ TxNxM1. Multivariate analysis in OAL of all histologic subtypes showed that the risk factors-associated PFS were positivity of BM involvement (Hazard ratio; HR=2.96, p<0.001 and HR>9.98, p=0.025, respectively), the risk factors-associated OS was non-MALT lymphoma subtype (HR=9.18, p=0.013). Then, subgroup analysis...
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/cIgM/D, TCL-1, MNSA, T-bet and IRTA-1. Gastroscopy with multiple biopsies was performed in 56 cases. FISH analysis for del7q was done in 13 cases.

Results: A symptomatic 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 MNSA 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%). 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 metastasize to it. Most cases remain stable, while few develop cytopenias due to an extensive BM infiltration. These latter cases apparently represent a distinct MZL category which requires further investigation.
Monitoring should be part of physician’s practice in these WM patients. Long-term toxicities are also seen, at similar rates and second cancers.

Summary/Conclusions: Anemia and B2M>3mg/l, suggesting future trials should focus on this subgroup. Two parameters decreased the duration of PFS2 with immunochemotherapy: predicted PFS and OS with good accuracy.

Background: Myelodysplasia. Second PFS upon salvage (PFS2) was available in 72 patients: RF significantly increased the risk of Richter, and CLB exposure the risk of interstitial pneumonitis>20% adversely impacted TTNT. IPSS scoring system did not improve prognostication. Long-term follow-up: 22% of patients had second solid cancers.

Results: Median follow up for the entire cohort is 6 years, median age at diagnosis 68.6y and at therapy 71.2y; 75% being above 65y at treatment. Significant differences between DRC/RF cohorts were: median age 74/64y, high IPSS score 63%/28%, B2M>3mg/l 74%/56%, DRC cohort: median PFS/Time To Next Therapy and Overall Survival were 33mo, 45.8mo and 78 at 5 years, respectively. Other reductions>20% had no impact on these outcomes, but age≥65y and anemia<11.5g/dl decreased PFS. Previous CLB therapy increased the risk for delayed toxicities (infections 39%, myelodysplasia 13% vs 16%, myelodysplasia 13% vs 3.8%), but not second cancers including Richter transformation. IPSS scoring system predicted PFS and OS with good accuracy. RF cohort: median PFS/Time To Next Therapy and Overall Survival were 53mo, 65mo and 90% at 5 years, respectively. Previous CLB had no impact on outcomes, but dose reductions>20% adversely impacted TTNT. IPSS scoring system did not improve prognostication. Long-term follow-up: 22% of patients had second solid cancers. RF significantly increased the risk of Richter, and CLB exposure the risk of myelodysplasia. Second PFS upon salvage (PFS2) was available in 72 patients: 47 DRC (PFS2 47mo), and 25 RF (PFS2 66mo), not significantly different. Only two parameters decreased the duration of PFS2 with immunotherapy: anemia and B2M>3mg/l, suggesting future trials should focus on this subgroup to challenge standard R-based regimens with rituximab.

Summary/Conclusions: We conclude that clinical trials results of DRC and RF are reproduced in our real-life cohort despite older ages, and high IPSS scores. Long-term toxicities are also seen, at similar rates and second cancers monitoring should be part of physician’s practice in these WM patients.

Infectious diseases, supportive care

P642 MICAFLUNOSIS VERSUS LIPOSOMAL AMPHOTERICIN B FOR EMPIRICAL ANTIFUNGAL THERAPY IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED TRIAL

Background: Invasive fungal infections (IFIs) incur significant morbidity and mortality in neutropenic patients with hematological malignancies (HEM) after chemotherapy. The risk for these infections is related to the intensity and duration of neutropenia, and varies from 2% to 40%. Mortality rates associated with documented IFIs are considerable, reportedly ranging from 30% to 60%. Empirical antifungal therapy is the standard care for neutropenic patients with HEM, who remain febrile despite broad-spectrum antibiotic treatment. Several antifungal agents including voriconazole (VRCZ) or liposomal amphotericin B (L-AMB) have been studied as empirical therapy for febrile neutropenia (FN). However, limited data are available concerning the efficacy and safety of micafungin (MCFG) in FN patients with HEM.

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. The efficacy rates of MCFG and L-AMB were not significantly different (38/72 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 (66/72 cases (91.7%) vs 59/66 cases (89.4%), P=0.855*), (4) absence of premature study drug discontinuation due to poor efficacy or drug-related adverse events (54/72 cases (75.0%) vs 47/66 cases (71.2%), P=0.615*), and (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 9/66 cases (13.6%), P=0.006*). 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*, 14/72 cases (19.4%) vs 34/66 cases (51.5%), P=0.001*). Chi square test.

Summary/Conclusions: MCFG was as effective as L-AMB, and better tolerated than L-AMB as an empirical antifungal therapy in FN patients with HEM.

P643 ANTIFUNGAL DRUGS INFLUENCE NEUTROPHIL EFFECTOR FUNCTIONS IN VITRO AND MODULATE PULMONARY DAMAGE IN INVASIVE ASPERGILLOSIS

Background: Antifungal agents like azoles, echinocandins or polyenes substantially contribute to reduced morbidity and improved survival of high risk patients in hematology. 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: 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), posaconazole (POSA), 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 dichlorofluorescein 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 i.n. with conidia of Aspergillus fumigatus. Afterwards, mice were analyzed concerning fungal burden and pulmonary damage (albumin ELISA) with neutropenic animals serving as controls.

Results: In vitro, pretreatment with POS led to enhanced activation (CD262L: 44% vs 18% at 13 +/- 2%, *; mean +/- SEM, p value ≤0.05 considered to be significant). Pretreatment with AMB showed increased expression of activation markers. Moreover, ISA impaired degranulation and phagocytosis in CD262L+ PMN (27% +/- 4 vs 44 +/- 1, LPS, *; as well as generation of ROS (22660 +/- 3286 vs 41190 +/- 2584, zymosan, *)), and IL-8 synthesis was substantially impaired. CAS showed an increased phagocytosis (75% +/- 6 vs 44 +/- 5, LPS, *), whereas degranulation and LPS triggered generation of ROS were reduced by trend. Pretreatment with conventional AmB resulted in activation of almost all effector functions besides impaired phagocytosis (43% +/- 3 vs 59 +/- 3, LPS, *). In contrast, LAmB did not significantly affect any effector function. IPA treatment in vivo resulted in reduced fungal burden as expected but lead to reduced albumin concentration in BAL (111 ng/ml +/- 46 vs 380 +/- 31, *) indicating a decreased pulmonary damage. Despite significant influence on PMN effector functions in vitro, MIC did not affect clinical course IPA in vivo.

Summary/Conclusions: CD101 and POS induce PMN activation, whereas ISA and MIC inhibit PMN effector functions in vitro. CAS shows variable modification on PMN. Possibly independent from its antifungal effects, POS reduces pulmonary damage in mice suffering from IPA in vivo. Further studies are needed to distinguish the obviously multidimensional immunomodulatory effects of different antifungal agents and to clarify their relevance in clinical practice.

P644
CHARACTERISTICS AND OUTCOME OF PULMONARY INFILTRATES IN ACUTE LEUKEMIA CLASSIFIED ACCORDING TO EORTC/MSG CRITERIA OF INVASIVE FUNGAL INFECTION: A PROSPECTIVE STUDY BY THE RETE EMATOLOGICA LOMBARDA
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Background: In acute leukemia (AL) patients (pts) pulmonary infections may be severe and worsen the final outcome of AL. They have been recently shown to adversely affect the outcome of bloodstream infections (BSI) in AL pts (Cattaneo et al. 2016). The radiologic characteristics of PI belong, according to the EORTC/MSG Study Group, to the diagnostic criteria of a pulmonary invasive fungal infection (IFI).

Aims: In order to better define the clinical and prognostic significance of PI in AL pts in a real-life setting, we have analyzed all PI diagnosed during consecutively feasible/infected episodes developing over a 26 months period in pts admitted to 9 hematologic institutions within the Reto Ematologica Lombarda (REL) network.

Methods: From Dec-12 to Jan-14, all febrile/infected episodes were recorded and data concerning PI extracted. PI were classified as specific and aspecific for IFI according to radiologic criteria.

Results: During 1086 episodes, 256 PI were diagnosed in 195 AL pts (MF 124/71; median age 60y; AML/ALL 163/32). PI incidence was similar during induction and relapse (28.8% and 29%, respectively), but significantly lower in complete remission (14.2%, p<0.0001). Overall, PI were detected in 57% of cases during AML induction/reinduction and in 44.5% during posaconazole prophylaxis. Posaconazole was not responsible for a decreased sensitivity of serum galactomannan (GM), which was positive in 18.4% and in 16.8% pts with specific PI receiving posaconazole or not, respectively. Aspecific PI were observed in 157 cases (61.3%). In the remaining 99 cases (38.3%) the specific radiologic criteria for suspecting IFI were met, but in 70 of them (27.3%) just in the context of a diagnosis of possible (pos) IFI. Probable/proven (prob/prov) IFI criteria were met in 29 PI (11.3%). The characteristics of the three subgroups of PI are listed in Tab. 1. Prob/prov PI IFI were associated with lack of posaconazole prophylaxis in comparison with poss IFI (72.4% vs 57.1%, p=0.0074).

Aspecific PI did not differ from poss IFI except for their lower frequency during neutropenia, particularly if 31/58 (80.3% vs 92.9%, p=0.0164, and 56.1% vs 80%, p=0.0053, respectively), and higher frequency in patients on Fluoroquinolone (Fq) prophylaxis (Fq, p=0.0192). Multivariate analysis confirmed that specific PI were less frequent during prolonged neutropenia (HR 0.382, IC 0.189-0.772), and poss IFI during Fq prophylaxis (0.344, 0.159-0.742). All but 21 cases requiring antifungal treatment during the study period were treated with Micafungin or Caspofungin. Thirty-day mortality was observed in 41 cases (16%). It was similar for aspecific and poss IFI (15.9% and 10%), but significantly higher in prob/prov IFI (31.3%, p=0.0192). Multivariate analysis confirmed a prob/prof IFI (3.277, 1.243-8.644) predictive for death, as well as relapsed/refractory AL (2.451, 1.092-5.498) and BSI (2.833, 1.006-5.377).

Summary/Conclusions: Among PI occurring in AL patients IFI could be suspected according to EORTC/MSG Study Group criteria in nearly 40% of cases but only 11% of PI met the criteria for prob/prov IFI. Posaconazole did not affect serum GM sensitivity and was protective against prob/prov IFI, which had a higher risk of death. On the other hand, PI associated with a diagnosis of poss IFI had a similar outcome compared to aspecific IFI, although they occurred more frequently during neutropenia and outside from Fq prophylaxis. These findings may be relevant in the context of a reevaluation of the criteria for suspecting IFI in AL patients with as well as for a more appropriate antimicrobial stewardship.

P645
ANTIFUNGAL PROPHYLAXIS WITH CD101 IN IMMUNOSUPPRESSED MOUSE MODELS OF CANDIDIASIS, ASPERGILLOSIS, AND PNEUMOCYSTIS PNEUMONIA (PCP)
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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 infection 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.

Methods: CD101 (5mg/kg) and Caspofungin (5mg/kg) were rendered neutropenic by cyclophosphamide (cpm) on day -4 (150mg/kg) and day -1 (100mg/kg) and challenged (day 0) with Candida albicans ATCC 53134 (IV, 100 µL, 105 CFU/mouse). One dose of CD101 5, 10, or 20mg/kg SC was given prior to challenge on day -5, -3, or -1. Kidneys were removed for CFU enumeration 24 h postchallenge. Aspecific PI were treated with systemic antifungal therapy. Thirty-day mortality was observed in 41 cases (16%). Aspecific PI were treated with systemic antifungal therapy. Thirty-day mortality was observed in 41 cases (16%). It was similar for aspecific and poss IFI (15.9% and 10%), but significantly higher in prob/prov IFI (31.3%, p=0.0192). Multivariate analysis confirmed a prob/prof IFI (3.277, 1.243-8.644) predictive for death, as well as relapsed/refractory AL (2.451, 1.092-5.498) and BSI (2.833, 1.006-5.377).

Table 1.
challenge on day -5, -3, or -1. Survival was monitored for 14 days. PCP model: C3H/HeN mice (10 g) were immunosuppressed by dexamethasone (40 mg/kg) in acidified drinking water and inoculated with Pneumocystis murina (intranasal- ly, 2 x 10^5/50 µL). CD101 0.2, 2, or 20 mg/kg intraperitoneally was given at the time of inoculation and 1 or 3x/wk for 6 weeks. TMP/SMX 50/250mg/kg/3x/wk was used as positive control. At 6 wks, lungs were processed for quantification of trophic and asci (cyst) forms of P. murina.

**Results:** Candidiasis: Kidney CFU decreased with higher doses of CD101 and shorter times between prophylaxis and challenge. At 20 mg/kg, there was complete clearance of CFU burden regardless of treatment day in all animals except one (prophylaxis on day -3). There was complete clearance in all animals given 10 mg/kg on days -3 and -1 and significant decreases in CFU in those given 5 mg/kg on days -3 and -1. Aspergillosis: Survival rates significantly increased following CD101 5, 10, and 20 mg/kg prophylaxis on day -5, -3 or -1 compared with vehicle. Prophylaxis closer to challenge increased the rate of survival in the CD101 group. All animals given higher doses survived regardless of day of prophylaxis. PCP: Trophic nucleus counts were significantly reduced versus untreated controls in all CD101 groups except 0.2 mg/kg/1x/wk, and efficacy in 3 different CD101 groups was comparable to TMP/SMX (no nuclei observed microscopically). Asci counts also were significantly reduced in all CD101 groups versus untreated controls. There was no significant difference in efficacy between TMP/SMX and CD101 in all but the lowest dose group (0.2 mg/kg/1x/wk), with no asci observed microscopically.

**Summary/Conclusions:** CD101, a novel echinocandin, was protective against fungal challenge in immunosuppressed mouse models of candidiasis, aspergillosis, and PCP. These data suggest that CD101 may provide benefit for acute leukaemias. Though optimised antifungal therapy might be effective, in selected patients, surgical interventions might be an useful tool both for diagnostic and therapeutic reasons. However due to the nature of the disease and circumstances, prospective data of Surgical interventions in these situations is very difficult and the evidence is usually from small cohorts often from single centres.

**Aims:** The purpose of this study is to report our single center experience of surgical interventions for IFI in acute leukaemia patients.

### Table 1.

<table>
<thead>
<tr>
<th>Organ involved</th>
<th>Infections (n = 10)</th>
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<tr>
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<td>liver</td>
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**Methods:** A retrospective review of our Hospital’s Leukaemia database (IRB approved) was made for clinical characteristics and outcomes in surgically managed IFI patients diagnosed between Jan 2005 and Dec 2015. IFI was defined by EORTC/MSG 2008 criteria.

**Results:** Among 795 acute leukaemia patients diagnosed during this period, we found 19 patients with IFI who had undergone surgical interventions (5 proven, 1 probable and 3 possible IFI). The details of the IFI, surgical interventions, antifungal 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 IFI patients had overall benefit from the procedure through optimization of antifungal therapy with MIC/sensitivities, arrest of aspergillosis related massive bleeding and/or complete resolution of the IFI 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 57.7 months (range 9 –118.3 months). Two patients who died also had benefited from the procedure and had survived for 6.5 and 47 months post-surgery but both succumbed to septic events unrelated to the IFI during subsequent chemotherapy. Of the remaining 6 patients (out of the 15 proven IFI), 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 IFI and 1 lacked information to draw any conclusions. The patient with probable IFI diagnosed 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 IFI, 2 were able to proceed with transplantation and 1 had chemotherapy post-surgery, 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 IFI. 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 especially for membrane-bound 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 P. notatum and whose dysfunction might be associated with higher Day +100 survival rates for all days, which was associated with increased Hb level. The safety results confirmed the safety profile of epoetin theta.

Methods: Genotyping of L-ficolin and PTX3 SNPs (rs17514136, rs17549193, rs17551936, and rs17549193, n=180) was performed by Taqman assay. Multiple logistic regression analyses were applied to evaluate the association between 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 rs17514136GG/AA or GG (n=100 or 22) revealed a significantly higher risk for developing sepsis (odds ratio [OR]: 1.88; 95% confidence interval [CI]: 1.01–3.27) or pneumonia (OR: 2.79; 95% CI: 1.1–6.9, p=0.01). A similar risk profile could be demonstrated for patients carrying rs17549193TT/CT 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)

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, rs17551936, and rs17549193, n=180) was performed by Taqman assay. Multiple logistic regression analyses were applied to evaluate the association between the polymorphisms and the occurrence of infectious events.

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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.

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 diagnosis at the start of defibrotide therapy may be associated with >80% mortality. Defibrotide is approved to treat severe hematopoietic VOD/SOS post-HSCT in the European Union and to treat hematopoietic 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 median of 22 days after VOD/SOS diagnosis. A total of 27 patients with VOD/SOS who developed VOD/SOS after chemotherapy had never received ESA prior to enrolment in this study and 45.2% benefited from the use of epoetin theta.: The latter group was started the day of diagnosis; in 89.7% (78/87), by Day 7. In the pooled chemotherapy subgroup, survival was analyzed from the day of diagnosis 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<.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<.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, Adamts13−/− 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 Adamts13−/− 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 different hierarchical origins.

Summary/Conclusions: 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.

P653
FUNCTIONAL STUDY ON THE COOPERATION OF ASXL1 AND RUNX1 MUTATIONS FOR LEUKEMIC TRANSFORMATION
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Background: Our previous studies showed that RUNX1 and ASXL1 mutations were frequently co-existed in chronic myelomonocytic leukemia (CMMML) (EHA 2015) and clonal evolution of RUNX1 and/or ASXL1 occurred most frequently in chronic myeloid leukemia (CML) with myeloid blast crisis (EHA 2016). The molecular pathogenesis of cooperation of RUNX1 and ASXL1 mutations has not been reported yet.

Aims: We aimed to determine the functional role of collaborative association of RUNX1 and ASXL1 mutations for secondary acute myeloid leukemia (sAML) transformation.

Methods: For in vitro study, we overexpressed RUNX1-WT/MT (R135T) in K562 cells which harboring ASXL1-MT (Y591K) and co-expressed with ASXL1-WT/MT (R693X) in murine 32D cells. After stable expression, functional properties were examined by using immunoblot, co-immunoprecipitation, quantitative RT-PCR, flow cytometry, cell proliferation, colony formation and gene expression microarray analyses. C57BL/6 mice were used for bone marrow transplantation (BMT) experiments for in vivo study.

Results: We found that RUNX1-MT augmented cell proliferation, colony formation, HOXA gene expression and inhibited megakaryocytic differentiation in ASXL1-MT K562 cells compared to RUNX1-WT or empty vector control. The cooperation of RUNX1 and ASXL1 mutations or the knocked down of ASXL1 cooperated with RUNX1-MT inhibited apoptosis and impaired differentiation in 32D cells. Nine months post BMT mice with the combined RUNX1 and ASXL1 mutations, but not RUNX1-MT or ASXL1-MT alone, developed disease characterized by marked splenomegaly, hepatomegaly, and leukocytosis with a shorter latency. We found that RUNX1-MT stabilized hypoxia-inducible factor 1α (HIF1α) gene and increased its target gene expression such as ID1 (inhibitor of DNA binding 1). Clinical samples analyses showed that ID1 expression increased in both RUNX1-MT and ASXL1-MT or the combined mutations of RUNX1 and ASXL1 compared to control samples. We also examined the impact of RUNX1 and ASXL1 mutations on sAML-free survival of 104 Patients with CMMML in whom 11 had co-occurrence of RUNX1 and ASXL1, 39 had either mutated ASXL1 or RUNX1 and 54 patients were negative for both mutations. We found that patients carrying co-existed mutations had a shorter sAML-free-survival (median 16.1 months, 95% CI 0.0-60.1 months) than those carrying either mutated gene alone (median 23.0 months, 95% CI 17.8-28.2 months) or negative for both mutated genes (median not reached, 59.2% ± 8.8% at 5 years) (P=0.023).

Summary/Conclusions: The present study demonstrated that clinical and functional evidence for a collaborative association of RUNX1-MT and ASXL1-MT for sAML transformation. We identified HIF-1α targeting a new pathway which may be critical for leukemic progression of RUNX1/ASXL1-mutated myeloid malignancies.

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 different hierarchical origins.

Summary/Conclusions: 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.

P654
A NOVEL MASS SPECTROMETRY METHOD REVEALS THE INTRACELLULAR PHARMACOKINETICS OF AZACYTIDINE THERAPY IN VIVO
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Background: The cytidine analog 5’-Azadecitidine (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 deoxycytidine 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 reduction reaction to reduce 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 demethylation. 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 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 the non-responders with the lowest levels of DNA-incorporated DAC. Additionally, in these non-responders, there was also concomitant increase in AZA incorporation into RNA.

Figure 1.

Summary/Conclusions: 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). Due to recent high-throughput sequencing studies, the mutational 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.cancergenomeinterpreter.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. TDS identified most recurrently mutated genes were SRSF2 (41%), TET2 (41%), STAG2 (28%), SF3B1 (21%), ASXL1 (21%), TPO3 (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 progression 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 type-1 mutations were detected in STAG2 gene. Thus, mutational burden of STAG2 were 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 SRSF2 (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.
Background: Progression of myelodysplastic syndromes (MDS) to acute myeloid leukemia (AML) associates with acquisition of genetic aberrations. Similar aberrations may occur in the development of primary AML, particularly in the context of a clonal hematopoiesis of indeterminate potential. Thus, in-depth knowledge of the genetics and clonal composition of MDS and paired AML provides all insights into MDS progression in particular and AML development in general.

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 by an NGS panel (Agilent HaloPlex, Illumina MiSeq) comprising 98 genes relevant in hematologic neoplasms, and for copy number variations (Affymetrix CytoScan HD). All AML have been matched with at least one single cell. Samples were collected during MDS, at AML diagnosis and under treatment. Variants were verified by Sanger and pyrosequencing or fragment analysis in BM and CD3+ cells (germline). Clonal assignment of variants was verified by single-cell mutation analysis using a Single-Cell Printer.

Results: Applied predefined criteria and verifying variants by orthogonal methods in blasts and CD3+ cells, a median of 3 variants (range, 1-6) in the MDS and 4 (range, 1-6) in the AML samples were deemed pathogenic. During MDS, all patients except one had mutations in genes involved in RNA-splicing (SF3B1, STAG2, CEBPA, RUNX1) or epigenetic regulation (TET2, DNMT3A, ASXL1, EZH2). Additional mutations existed in FLT3, NRAS, PTEN, STAG2, CEBPA, RUNX1 or WT1. Subclonal mutations (i.e. variant allele frequency (VAF) <1%) were present in only two MDS samples. Towards AML, patients acquired a median of 1 (range, 0-2) new mutation in FLT3, CSF3R, KRAS, NRAS, PHF6, IDH1 or WT1. The VAF shifts from MDS to AML indicated cooperativity of mutations on clonal outgrowth. e.g. gain of CSF3R p.T618I was accompanied by a chromosome 19q-loss resulting in hemizygosity of a preexisting CEBPA mutation; or acquisition of a FLT3-TKD mutation was associated with outgrowth of a RUNX1 mutation. Changes in mutations or VAFs also occurred in single cells, e.g. in one AML sample under decitabine treatment by gaining two distinct FLT3 mutations. In another patient, who achieved complete remission after induction chemotherapy, but relapsed with MDS, which again progressed to AML, mutations were lost or gained, while a STAG2 mutation was detectable at all time points. Interestingly, identical genes were recurrently mutated in different clones with single patients, e.g. progression to AML associated with acquisition of a WT1 mutation in an NRS mutated MDS clone and with the generation of further subclones harboring distinct combinations of different WT1 and NRAS mutations. The co-occurrence of the specific WT1 and NRAS mutations in the different clones was demonstrated by mutation analyses of 72 single patient cells.

Summary/Conclusions: Mutations in MDS are few in number, but enriched in genes involved in RNA-splicing or epigenetic regulation; gain of single driver mutations leads to clonal outgrowth and thus, AML. Subsequent treatment can change the mutational and clonal profile, in particular, identical mutations were found in different clones, as confirmed by single-cell analyses; this suggests a fertile ground (e.g. microenvironment) for such mutations in a patient and may lead to (a therapeutically exploitable) competition of clones.

Figure 1.

Summary/Conclusions: Although the in vivo 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 M5S) 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 model to test therapeutic effects of different drugs and other approaches on the MDS clonality and autologous MScs prior to treatment of MDS patients.

P656

MYELODYSPLASTIC SYNDROMES WITH IRON OVERLOAD ARE CHARACTERIZED BY A SWITCH FROM OXIDATIVE PHOSPHORYLATION TO GLYCOLYSIS AND THIS DEFECT IS PARTIALLY RESTORED BY IRON CHELATION. A FISIM STUDY


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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by a clonal and ineffective hematopoiesis, as well as the tendency to develop iron overload, mainly due to red blood cell transfusions. Iron overload has been described to increase ROS production and progressively worsen hematopoiesis. In mitochondria, iron is a fundamental component of cytochromes belonging to the oxidative phosphorylation (OXPHOS), which is considered the main source of cellular energy. Mitochondria are also the main site of ROS production. In this regard, cancer energetic metabolism is an emerging issue that could represent an attracting therapeutic target.

Aims: The aim of the study was to investigate the energetic metabolism in MDS patients and to understand the impact of iron overload on the energy production.

Methods: We selected 37 samples from patients with MDS with or w/o iron overload (7 RA, 5 RARS, 9 RCM, 4 RAEB-I, 2 RAEB II and 10 sAML). In addition we analyzed 86 samples from healthy subjects stratified according to the iron status, the OXPHOS activity, in term of oxygen consumption and ATP synthesis, the lactate dehydrogenase (LDH) activity, and malondialdehyde (MDA), as marker of lipid peroxidation. The same parameters have been analyzed also after iron chelation with deferasirox (DFX) and after incubation of the cells with DFX and DFO.

Results: Our study clearly demonstrated that mitochondrial function is altered in MDS, leading to a strong energetic defect and an increase in oxidative stress, far beyond the expected parapathophysiological decrease resulting from ageing. The OXPHOS efficacy is highly reduced in MDS compared to controls, deter-mining an impairment of the ATP/AMP ratio, which is 2.4 in young controls, 0.75 in elderly controls and it is 0.2 in b-thalassemia and MDS patients. By contrast, LDH activity increased in the MDS patients (6mU/mg) with respect to...
the controls (88 μM/μg), suggesting an attempt to compensate the energy unbalance with the increment of anaerobic glycolysis. MDA level, which reflects the lipid peroxidation, is 1 mM in young subjects, 9 mM in elderly subjects, 9 mM in b-thalassemia and 15 mM in MDS. In vitro iron chelation partially restored this abnormalities in MDS patients: ATP/AMP ratio increases from 0.2 to 0.6 in MDS and b-thalassemia, by contrast it is reduced in healthy subjects from 2.4 to 1.6. Anaerobic glycolysis is reduced after DFX incubation, in fact LDH decrease from 88 to 77 in MDS. By contrast, in healthy samples the iron chelation determined a reduction of OXPHOS activity, with a consequent impairment of ATP/AMP ratio and an increment of anaerobic glycolysis flux. Lipid peroxidation is significantly reduced by 28% with DFX and 23% with DFO (p value <0.001 for both). Similar reduction is observed in b-thalassemia. By contrast MDA levels increased in healthy subjects incubated with DFX. Curiously, all these abnormalities are more pronounced in MDS with IOL compared to MDS w/o IOL and are significantly worse in MDS without IOL compared to elderly non-iron overloaded. Furthermore, the expression of patients with DFX reproduces similar findings as in vitro incubation.

**Summary/Conclusions:** In summary OXPHOS activity and the energetic status are highly impaired in MDS compared to elderly subjects. MDS cells used O2 to produce ROS instead of ATP. This is typical of ageing but is significantly increased in MDS compared to elderly controls and it is further increased by IOL. DFX is able to restore mitochondrial activity and ATP production in all the patients analyzed after in vivo or in vitro treatment.

**P659 V-SET AND IMMUNOGLOBULIN DOMAIN-CONTAINING 4 (VSIG4) EXPRESSED ON MONOCYTES INCLUDING TUMOR-ASSOCIATED MACROPHAGES SUPPRESSED ANTITUMOR IMMUNE RESPONSES IN MYELODYSPLASTIC SYNDROMES**

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**Background:** In myelodysplastic syndromes (MDS), blast cells increase with clonal proliferation during disease progression, while immune cells in the bone marrow (BM) microenvironment become less efficient. The V-set and immunoglobulin domain-containing 4 (VSIG4) molecule is a new B7 family-related protein and strong negative regulator of T-cell proliferation. However, the role of VSIG4 in tumors including hematological malignancies remains unknown.

**Aims:** We investigated the expression and functions of VSIG4 in MDS.

**Methods:** 1) Peripheral blood (PB) and BM samples were obtained from 39 patients with acute leukemia transformed from MDS (AL-MDS, N=21), MDS (N=13), and chronic myelomonocytic leukemia (CMMML, N=5) and from healthy controls (N=14). The expression of VSIG4 in mononuclear cells (MCs) from the samples and MDS cell lines (F-36P and SKM-1) was analyzed with real-time PCR and flow cytometry (FCM). 2) After cultivation with BM stromal HS-5 cells, its culture supernatants (HS-5 sup.), immunomodulatory drugs (lenalidomide [LEN] and pomalidomide [POM]), and anti-MDS agents (cytarabine and azacitidine), we validated them by RT-PCR in an extension cohort of 73 additional MDS patients and assessed their potential singular pattern in this disease by analyzing 80 MDS and 90 AML patients. Further we chose 13 of the differentially expressed genes for validation and characterization through the myeloid spectrum based on clinical conditions: i) druggable oncogenes found to be overexpressed in our cohort; ii) oncogenes found in our patients allowing for the inhibition of an specific DNA repair pathway (i.e. XPA, XRCC4, MSH4); iii) oncogenes infra-expressed in our cohort but with inhibitory molecules already being tested in myeloid neoplasms (i.e. PARP1). Global pattern of DNA repair gene expression was compared with MDS and AML MILE study data.

**Results:** Of 27 CMML patients and 10 healthy donors, the expression of 18 genes was significantly different between the two groups (p-value<0.05) with 6 genes up-regulated and 12 genes down-regulated in CMML patients compared with donors. Defects on genes uniquely predominant to a single leukemia were found: i) druggable oncogenes found to be overexpressed in our cohort, ii) oncogenes found in our patients allowing for the inhibition of an specific DNA repair pathway (i.e. XPA, XRCC4, MSH4): iii) oncogenes infra-expressed in our cohort but with inhibitory molecules already being tested in myeloid neoplasms (i.e. PARP1). Global pattern of DNA repair gene expression was compared with MDS and AML MILE study data.

**Conclusion:** Using an unbiased and massive DNA repair transcriptome assessment, we have identified a series of candidate targets for a synthetic lethality approach in CMML. In addition, the different sense of misregulation of these and other targets within the myeloid diseases, some of them already being targeted in the clinical trial setting, emphasize the need of a new plasm-personalized test of DNA repair modulators.
on cytomorphological characteristics, but it remains a challenge in some patients who do not fulfill diagnostic criteria. Flow cytometry (FC) immunophenotyping can be an important tool for MDS diagnosis, but a lack of standardization and subjectivity of the analysis hinders its applicability.

**Aims:** To develop a methodology for FC immunophenotyping that allows us to establish the differential diagnosis between MDS patients and non-clonal cytopenias using a myeloid maturation database.

**Methods:** Bone marrow samples from 55 MDS patients, and 51 controls with cytopenias of several origins (immune disease, hypersplenism, drug toxicity) were analysed by FC. We elaborated a Myeloid Maturation Database using the Infinicyt® software (Cytognos, Spain). From all bone marrow controls, we merged files stained with a 4-colour combination (CD16-FITC/CD13-PE/CD45pCP/CD11bAPC). We selected myeloid population from the merged file and drew a maturation path. We obtained a maturation diagram that displays the fluorescence intensity of each parameter measured along the maturation stages. Then, for patients and controls, we obtained the fluorescence intensities whose median values exceeded ±2SD range in comparison with the stored database values (Figure 1). We elaborated a score, considering the relevant changes in fluorescence intensities (deviations) in the four markers analysed (CD16, CD13, CD45, CD11b) and in the four maturation stages, with a punctuation from 0 to 16.

**Results:** We found a mean of 1.9 deviations (fluorescence intensities values exceeded ±2SD) in controls, and a mean of 4.5 deviations in patients. Our test resulted reliable for differential diagnosis between controls and patients (curve ROC analysis, AUC = 0.748; p = 0.016). We found that with a cut-off of 4.5 deviations, we obtained a high specificity in the diagnosis of MDS (100%) but a low sensitivity (45%). With a high suspicion of MDS specificity (90%), we can consider patients with scores above 3.5, thus achieving higher sensitivity (59%). Additionally, the number of immunophenotyping changes correlated well with prognostic risk. We confirmed that the higher the score, the greater impact on deviations from the normal pattern (average of 3.7 at low risk, 4.5 at intermediate risk; 6.8 at high risk) (Figure 2).

**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 sensitivity in differential diagnosis.
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 a Kaplan-Meier analysis. Multivariate Cox regression analyses were performed.

Results: 2035 patients (RA n=182, RCMID n=978, RARS n=170, MDSde5q n=92, RAEAB-12 n=613) with a median follow-up of 23 months (mo) were included. 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 17.9 vs. 26.4 months, Log Rank p < 0.001). 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 of borderline significance (median 67 vs 47 months, Log Rank p=0.1, Breslow p=0.39). 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 vs 43.0 months, Log Rank p=0.002). This was not the case in the other IPSS-R subgroups. In multivariable analyses, an independent adverse impact of lymphopenia (defined with a lymphocyte count <850/μl) was confirmed in 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 platelets (median 97 versus 150 G/l, p=0.001) and neutrophiles (median 1478 versus 1971/μl, p<0.001) counts but similar haemoglobin levels (median 9.4 g/dl versus 9.4 g/dl, p=0.052).

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 related 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.
start of treatment was 21 months (95%CI=19-24); CR: 25 months (95%CI=20-30); PR: 27 months (95%CI=20-30); and SD: 17 months (95%CI=14-19) (p=0.006). We compared OS between mCR vs PR (p=0.193, HR 0.796 [95%CI=0.765-1.122]), mCR vs PR (p=0.572; HR =0.564 [95%CI=0.378-0.845]) or mCR vs SD (p=0.243; HR=1.242 [95%CI=0.863-1.788]), without any statistical difference (Fig. 1A). Median progression-free survival (PFS) was 14 months (95%CI=13-16); CR: 16 months (95%CI=13-21); PR: 11 months; mCR: 10 months (95%CI=5-15); and SD: 10 months (95%CI=9-12) (p=0.013). No statistical differences were observed between PFS in patients who achieved mCR vs PD (p=0.410; HR 1.816 [95%CI=0.439-7.512]) and SD (p=0.7743; HR 1.059 [95%CI=0.752-1.491], but PFS was increased in those patients who achieved CR when compared to mCR (p=0.013; HR 0.665 [95%CI=0.482-0.918]) (Fig. 1B).
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 recorded 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 cytopenias or the cytopenias after AZA 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%), 115 as 2nd line treatment (26%), and 34 as a line ≥3rd (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%) A-complete (n=126, 30%), A-EB2 (n=189, 45%), other subtypes (n=15, 4%). At start of AZA therapy IPSS score was low in 14% (3.4%), int-1 in 9% (23.2%), int-2 in 16% (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 with 72% achieving a complete hematological response, 77% (22%), a partial response, 86% (25%) had stable disease while 36% (40%) 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 had been discontinued. Interruption of treatment was due to loss of response in 59 patients, all of them with AML evolution in 154 (40%), death in 43 (11%), toxicity or poor compliance in 39 (10%), allogeneic HSCT, 27 (7%) low-dose chemotherapy (7%), 22 (6%) erythroid stimulating agents, 18 (5%) other treatments and 272 (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 syndromic 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 treatment, 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 Covemri software. Library preparation was performed using an illumina Truseq Custom Amplicon panel, followed by sequencing on an illumina Miseq. Data analysis was performed using our established bioinformatic pipelines (Hamblin 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: MPN/JMML (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, RAD pathway mutations were common in the JMML/MPN (100%) and de novo MDS patient subgroups (33%). Additional mutations in epigenetic modifiers, splicesome mutations as well as second RAD 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, TET3). In contrast to the other MDS/MPN cases, in this group, no RAD 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 of 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 RESPONSES TO ASCT (PADIMAC)


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Background: The role of autologous stem cell transplantation (ASCT) as first line therapy for newly diagnosed (ND) patients with multiple myeloma (MM) remains under investigation despite the demonstrated PFS and OS benefits for ASCT patients. Outcomes for those not proceeding to ASCT following induction remain unclear, likely to be influenced by genetic risk and response depth. This study was designed to evaluate a stratified approach to ASCT, investigating if patients not proceeding to ASCT 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 further treatment. Here we report the primary stratification data 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 days 1, 4, 8, 11; lenalidomide 15mg days 1-8 and 15-21; dexamethasone 40mg days 1-4 and days 8-11 and 15-18 for cycle 1 only) for 4-6 cycles. Those achieving <PR were off protocol; all others had PBSC followed by restaging including MRD assessment on bone marrow using multi-parameter flow cytometry. Those in PR were stratified to ASCT (no maintenance) whereas those achieving ≥VGPR stopped induction therapy and were assessed at 100 days post PBSC (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). Response rate alone is not sufficient to identify patients who would benefit from ASCT and use of MRD to stratify treatment is now being investigated.

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 standard risk and 43 (32.6%) were adverse risk. 51 (33.6%) patients were ISS I, 67 (44.1%) ISS II and 34 (22.4%) ISS III. 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: ≥ 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. After a 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 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=11) patients at D100 post-PBSC respectively, 2+y-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+y-PFS 25.0% (95% CI: 0.9-42.2) and 42.9% (95% CI: 6.2-79.6) 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 not proceeding to ASCT. The median PFS for patients with adverse risk FISH lesions was significantly shorter than standard risk patients (p=0.008). 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: 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.
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 AOU 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%) or OCR (35%) was administered.

Results: 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 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: 87% vs 23% 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 allows the possibility of a “whole body” analysis in exchange for higher “biologic” cost.

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 (PCY-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-risk 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 nausea (50%), upper respiratory tract infection (30%), and asthenia, peripheral edema, hypocalcemia and hypokalemia (20% each). The most common Grade ≥3 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.
Results: There were 78 patients in Group 1 and 52 patients in Group 2. Patients in Group 2 had higher baseline dFLC, 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 or undetectable cPCs before transplant, including organ involvement, baseline dFLC, 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>
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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 with 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 not found to 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

RENA L IMPAI RMENT IN MYELOMA - PATIENT CHARACTERISTICS, TREATMENT MODALITIES, STEM CELL TRANSPLANT & OUTCOMES FROM THE AUSTRALIAN AND NEW ZEALAND MYELOMA REGISTRY

Results: Of 867 patients, 775 had eGFR available at diagnosis: 34% (287/775) had eGFR <60ml/min (22% at 30-60ml/min; 6% at 15-30ml/min; 6% at <15 ml/min). Mean age of patients with RI (<60 ml/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 ml/min compared with 8% >30ml/min. Patients with RI (<30ml/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), amp1q21, del13q) & high LDH were more prevalent in RI vs 46% (p=0.01). It was advanced stage (ISS III) (66% vs 12% p<0.001). 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 <60ml/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 <15ml/min had better OS & PFS compared with eGFR 15-30ml/min; dialysis in eGFR <15ml/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 <15ml/min. In patients with eGFR <60ml/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 (<30ml/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. 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 <15ml/min had a better OS than 15-30 ml/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 <30ml/min), supporting the benefit of ASCT in MM patients with RI.

P675

VENETOCLAX AS TARGETED THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA

Results: Venetoclax (VEN), an orally available selective small-molecule BCL-2 inhibitor, has shown activity in MM patients with high-risk features, and was associated with improved outcomes in several studies.数据表明，Venetoclax（VEN）是一种口服的、选择性的小分子 BCL-2 抑制剂，在 MM 患者中显示出活性，并且在具有高风险特征的患者中与改善的结局相关。
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 up to 13 (range: 1–15); 46 (70%) pts were refractory to bortezomib, 20 (30%) to carfilzomib, 51 (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 1.35 months). Fifteen (23%) pts discontinued, and 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 VGPR). For all pts, median time to progression (TTP) and median duration of response (DOR) were 7.8 and 9.7 months, respectively. A clear difference in responses was seen among pts with t(11;14) vs without [ORR, 40% vs 6%; nVGPR, 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. A high BCL2:BCL2L1 (BCL-X) gene expression ratio was observed in 10/44 (23%) baseline tumor samples, enriched in pts with t(11;14) compared with non-t(11;14) (38% vs 5%) and associated with clinical response; 80% (8/10) of pts [all t(11;14)] with a high BCL2:BCL2L1 ratio achieved ≥PR with a median TTP of 11.5 months. Among pts with t(11;14) who were refractory to the last therapy, ORR was 42% (11/26); for t(11;14) pts refractory to both bortezomib and lenalidomide, ORR was 33% (8/24). For t(11;14) pts refractory to both bortezomib, lenalidomide, and pomalidomide, ORR was 40% (8/20) and 50% (3/6), respectively. No difference was seen in ORR for t(11;14) pts with high-risk del(17p) versus those without the deletion [40% (2/5) vs 40% (10/25)].

**Summary/Conclusions:** VEN has an acceptable safety profile with promising single-agent anti-myeloma activity in pts with R/R MM positive for t(11;14) who failed multiple prior lines of therapy.
dependent association with PFS was found for SKY92, r-ISS, deletion of 13q and t(11;14) (Table 1). A bidirectional stepwise selection procedure was applied excluding covariates with the highest $p$-values until only significant covariates remain. Initially included covariates were SKY92, r-ISS, gain(1q), del(17p), del(13q), t(4;14), t(11;14), LDH and age. ISS could not be included as it was highly correlated with other covariates (i.e. co-linear); t(14;16) was excluded due to the minimal number of present instances. HR: hazard ratios relative to the lowest risk category with 95% confidence intervals (CI), $p$: likelihood ratio $p$-value indicating the association of each covariate with OS.

Table 1. Multivariate survival analysis in the HO87/NM18 trial.

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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 comorbidity 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), paralysis (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).

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.

Multiple Myeloma and Comorbidity: A Population-Based Study

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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 1985 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. Comorbid 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: Among all patients, 55% had no prior history of comorbidity, 23% had one comorbidity, 12% had two comorbidities, and 10% had three or more comorbidity conditions. Survival was negatively influenced by the number of comorbidities. 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), paralysis (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).

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.
Madrid, Spain, June 22 – 25, 2017

Myeloma and other monoclonal gammopathies Clinical 4
P679

DETECTION OF NEW EMERGING CLONES DURING TREATMENT BY NGS
ALLOWS A BETTER RISK PREDICTION ON MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is a genetically complex disease, characterized by the presence of multiple clones with differing degrees of drug sensitivity at the time of diagnosis. Consequently, therapeutic response of MM
patients is unpredictable and extremely variable, and although the treatments
introduced over the last decade have significantly improved the outcome of
these patients, most patients eventually relapse. Deep sequencing methods
have contributed to increase the knowledge about the clonal heterogeneity of
the disease and helped to stablish the three evolution patterns at relapse: linear
and branching clonal evolution, and no clonal changes.
Aims: To analyze the diversity and relative dominance of different clones and
their evolution throughout the course of disease by NGS of the immunoglobulin
repertoire in MM patients. To evaluate if the presence of different clones is
associated with increased risk.
Methods: Immunoglobulin repertoire was analyzed by NGS in bone marrow
samples from 180 MM patients included in three GEM clinical trials
(NCT00461747, NCT00443235 and NCT01237249). The two first clinical trials
involve patients younger than 65 years old, and were analyzed with ClonoSeq
methodology, the later one involve patients older than 65 years old, and were
analyzed with a local NGS method recently validated (Martinez-López et al,
Laukemia 2017). A clonotype was identified when at least 400 identical
sequencing reads were obtained, or it is present at a frequency of >1%.
Results: Of the 180 MM patients studied, 57 (32%) shows the presence of
more than one clone throughout the clinical the course of the disease. The
identification of new evolving clones was only possible in the GEM10 clinical
trial with the Local NGS method; in this clinical essay, 6% (4/71) of patients
shows the development of different clones during treatment. We show that the
frequency of the predominant clone at diagnosis of these four patients
decreased with treatment, but the frequency of the new ones increased and
the patients progressed. When more than one clone is present at diagnosis,
the relative dominance of the clones varies throughout the course of disease
in an independent manner. There were no differences in median MRD values
between patients with one clone or more than one clone (0.0082% and 0.0055%
respectively). The presence of more than one clone was not associated with
high-risk cytogenetics. The presence of more than one clone at diagnosis does
not condition the prognosis in any of the patients and treatments analyzed.
Median PFS was 38 and 58 months for patients with one clone or more one
clone, respectively (HR=1.136, p= 0.563). Median OS was not reached for
patients with one clone, and was 81 months for patients with more than one
clone (HR=1.43, p=0.28).
Summary/Conclusions: The analysis of the IG repertoire by the local NGS
method during treatment is able to identify and quantify new emerging clones
during the treatment that were not detectable at diagnosis. The new clones
contributed to increase the MRD levels in the follow-up samples.The presence
of of different clones at diagnosis is not associated with higher risk of progression, high risk cytogenetics or higher MRD values.
P680

FINAL RESULTS OF PHASE (PH) 1/2 STUDY OF CARFILZOMIB,
POMALIDOMIDE, AND DEXAMETHASONE (KPD) IN PATIENTS (PTS) WITH
RELAPSED/REFRACTORY
MULTIPLE
MYELOMA
(RRMM):
A
MULTI-CENTER MMRC STUDY
1,*
1
1
2
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Background: In the era of increased use of 1st-line and maintenance lenalidomide (LEN), there is growing need for effective ≥2nd-line therapies (tx) for LEN-

refractory pts. The combination of carfilzomib (CFZ), pomalidomide (POM),
and dexamethasone (DEX) has shown promising activity in advanced RRMM,
including for pts refractory to LEN (Shah et al. Blood. 2015).
Aims: In this Ph 1/2 study, KPd was evaluated in RRMM, with a focus on pts
who are LEN-refractory but proteasome inhibitor (PI)-naïve/sensitive.
Methods: LEN-refractory disease was required for 2nd-line KPd and LENrefractory/exposure for ≥3rd-line. Ph 1 dose escalation to determine maximum
tolerated dose (MTD) followed the TITE-CRM design with 40 pts receiving 3–
4mg POM PO (days [d] 1–21), 20–36mg/m2 CFZ (IV d1, 2, 8, 9, 15, 16 in cycles
[C] 1–8, and d1, 2, 15, 16 in C9+), and 40/20mg DEX PO weekly (C1–4/5–8;
for all dose levels [DLs]) in 28-day cycles. CFZ was started at 20mg/m2 for d1,
2 of C1 for all DLs. Per design, 30 PI-naïve/sensitive pts were to be enrolled at
MTD across Ph 1/2, with partial response (PR) rate after C4 as 1º efficacy endpoint.
Results: As of 12/1/16, 65 pts have been enrolled, with efficacy and safety
data available for 64 pts: 4 at DL1 (20mg/m2 CFZ, 3mg POM), 29 at DL2
(20mg/m2 CFZ, 4mg POM), 32 at DL3 (20/27mg/m2 CFZ, 4mg POM), 0 at DL4
(20/36mg/m2 CFZ, 4mg POM). Median age was 63 y, median time from diagnosis 5.1 y, median prior tx lines 2, and 94% had refractory disease. Cytogenetic
data were available for 59 pts; 33% were high risk per IMWG. There were 9
dose-limiting toxicities, all asymptomatic cytopenias: 8 pts with grade (G) 3
neutropenia and 1 with G4 thrombocytopenia. The MTD was established at
DL3. In 64 pts, G3/4 hematologic toxicities included neutropenia (25%) and
lymphopenia (14%), and non-hematologic toxicities (all grades) included fatigue
(51%), dyspnea (42%), and gastrointestinal (45%). PRs were rapid with a ≥PR
rate of 63% after 1 cycle and 77% after 4 cycles. After a median of 20.9 cycles
(range, 1–49), ≥minimal response was 95%, ≥PR 84%, ≥very good PR 52%,
and ≥near complete response (nCR) 20%. In the 1º population (N=55, 51 LENrefractory, 28 progressing on LEN maintenance), ≥PR was 84% with 30 treated
at MTD. After median follow-up of 21 (1–49) mo, median progression-free survival (PFS) for all 64 pts enrolled was 16.8 mo and 2-y overall survival (OS)
was 76.8% with 20 pts remaining on treatment. For standard-risk (n=38) vs
high-risk pts (n=21), ≥PR was 89% vs 81%, ≥nCR was 24% vs 10%, median
PFS was 22.3 vs 10.6 mo, and 2-y OS was 90.8% vs 56.0%.
Summary/Conclusions: KPd is well tolerated and highly active (≥PR 84%)
with encouraging PFS (median 16.8 mo) in an RRMM pt population that was
mostly LEN-refractory and PI-naïve/sensitive. The results support planned evaluation of KPd with daratumumab in RRMM, particularly for high-risk pts.
P681

PANOBINOSTAT INDUCES CD38 UPREGULATION AND AUGMENTS THE
ANTI-MYELOMA EFFICACY OF DARATUMUMAB
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Background: Immunotherapy with the anti-CD38 monoclonal antibody (mAb)
daratumumab is increasingly being utilized in myeloma patients with
relapsed/refractory (R/R) disease after prior treatment with immunomodulatory
drugs (IMiDs) and proteasome inhibitors (PIs). However, the efficacy of daratumumab is limited by low CD38 expression on myeloma cells. Here, we investigate the use of the histone deacetylase inhibitor (HDACi) panobinostat to
modulate target antigen expression on myeloma in favor of potent mAb-mediated recognition and destruction. We show that panobinostat augments CD38
expression specifically on myeloma cells and demonstrate powerful synergy
with anti-CD38 mAb daratumumab.
Aims: Determine the impact of panobinostat on upregulation of CD38 expression on myeloma cells in order to enhance the efficacy of daratumumab.
Methods: Myeloma cells were treated with titrated doses of panobinostat (0,
10, 25 nM) and expression of CD38 and a panel of additional target molecules
including SLAMF7, as well as accessory ligands analyzed by flow cytometry at
24, 48 and 72 hours. Antibody-dependent cellular cytotoxicity (ADCC) against
panobinostat treated and untreated myeloma cells was analyzed at 4 and 20
hours after addition of PBMC at an effector to target ratio of 25:1 in the presence
of daratumumab or an isotype control antibody.
Results: We treated primary myeloma (n=12 patients) with panobinostat (10
vs 25 nM) and observed a uniform increase in CD38 expression in each case
by flow cytometry. Upregulation of CD38 was already detectable after 24 hours,
peaked after 48 hours of exposure to panobinostat and was higher at the 25
nM compared to the 10 nM dose. At 48 hours, the mean fluorescence intensity
(MFI) for CD38 expression was 4 (10nM) and 6-fold (25 nM) higher in panobinostat-treated compared to untreated myeloma (p<0.01). The increase in CD38
was equal in patients with previously untreated (n=5) and R/R myeloma (n=7);
and could be confirmed in a panel of myeloma cell lines, including MM1.S and
OPM-2. The panobinostat-induced upregulation of CD38 was rapidly reversible
after drug withdrawal. Further, the increase in CD38 expression after panobinostat treatment was specific for myeloma and neither observed this phenomenon in a panel of leukemia and lymphoma cell lines, nor on primary CD8+
and CD4+ T cells that we isolated from peripheral blood of several donors

haematologica | 2017; 102(s2) | 271


and del(17p). as determined by interphase FISH analysis, including t(11;14), t(4;14), del(13q). Responses in high achieving a VGPR or better. Median TTP (11.6 months). Sixteen of 27 patients with low (66%) achieving VGPR or better (p<0.01). The synergistic anti-myeloma efficacy of panobinostat and daratumumab was confirmed with a panel of myeloma cell lines.

Summary/Conclusions: Our data demonstrate that the HDACi panobinostat induces upregulation of CD38 on myeloma and a subsequent dramatic increase of daratumumab-mediated ADCC. These data suggest that panobinostat could be used synergistically with daratumumab in a clinical setting to increase response rates and extend duration of responses to daratumumab.

P682

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 promote myeloma cell survival. Venetoclax (VEN) is a potent, selective, and orally bioavailable small-molecule inhibitor of BCL-2. Bortezomib (BTZ) is a proteasome inhibitor that can inhibit MCL-1 activity by increasing the MCL-1 antagonist, NOXA.

Aims: Results presented herein describe correlative biomarker analyses in the ongoing phase 1b study of VEN in combination with BTZ and dexamethasone in patients with relapsed/refractory (R/R) MM (NCT01794507).

Methods: As of 19 Aug 2016, 86 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 vs 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 with a high response rate. On all, 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 4.7 months) were significantly longer in patients with low BCL2 expression. Responses in high BCL2 expressors 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, type and switches of RRT or hospitals, 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 1.3–3.3), Fig. 1). In addition, patients who started dialysis in the period after bortezomib was introduced became dialysis independent more rapidly than in the pre-bortezomib period (1.2 compared to 1.7 years; p < 0.001). Age < 75 years (vs. ≥ 75 years) and light chain deposition disease (LCDD) as the primary renal disease (vs. amyloidosis) were significantly associated with achieving dialysis independence (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.

P684

TREATMENT WITH POMALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH MULTIPLE MYELOMA AND LIGHT CHAIN (AL) AMYLOIDOSIS

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Background: The immunomodulatory agent pomalidomide is active in patients with relapsed/refractory multiple myeloma, including those who failed prior lenalidomide and bortezomib. Phase II clinical trials showed that pomalidomide is also effective in primary AL amyloidosis. After this drug was marketed for multiple myeloma (in Italy since September 2015), it became routinely accessible also to patients with myeloma-associated AL amyloidosis, a particularly fragile population.

Aim of this study is to report the efficacy of pomalidomide and dexamethasone in patients with multiple myeloma-associated AL amyloidosis.

Methods: The databases of the Pavia Amyloid Research and Treatment Center were searched for patients with a diagnosis of multiple myeloma and AL amyloidosis who underwent an autologous stem cell transplant and/or previous pomalidomide treatment and at least partial response or better was achieved. A total of 358 patients were used (median age 66 years; 273 were men). Heart involvement was present in 13 patients (43%) and kidney involvement in 18 (60%). Four (13%) patients were in Mayo Stage I, 17 (57%) in stage II and 9 (30%) in stage III. Fifteen (50%) subjects were in renal stage I, 8 (27%) and 3 (23%) were in renal stage II and I and respectively and 5 (16%) patients were on dialysis at the time of P Dex initiation. Median bone marrow plasma cell infiltrate was 20% (range: 10-90%). Twenty-three (76%) patients were refractory to all previous lines of therapy. Median time from diagnosis to transplant was 6 months [interquartile range (IQR): 3-20 months]. Adverse events were observed in 5 (17%) 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 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 previously thalidomide-based regimens. The median number of P Dex cycles performed was 4 (range: 1-11). Median follow-up of living patients was 6 months [IQR: 3-15 months]; 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 responses were observed in 1 of 5 patients with measurable NT-proBNP (20%), but this can be underestimating 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.

P685 MYOCARDIAL UPTAKE OF 99mTc-DPD IN PATIENTS WITH AL AMYLOIDOSIS

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Background: AL amyloidosis is a light chain (FLC) deposition disease that can affect the heart. To identify the specific subtype is essential for treatment and prognosis. 99mTc-DPD scintigraphy (SC) has shown high sensitivity and specificity for detecting TTR cardiac amyloidosis, although some cases have been described with AL amyloidosis and positive 99mTc-DPD SC. We did not find any correlation between positive cardiac uptake in the SC and NT-proBNP values, as it has been recently suggested.

Methods: Methods: We performed 704 SC for different indications during the study period. In 220/319 (69%) MM patients treated with induction chemotherapy, SC was performed for patients with relapsed/refractory multiple myeloma, including those who failed prior lenalidomide and bortezomib. Phase II clinical trials showed that pomalidomide is also effective in primary AL amyloidosis. After this drug was marketed for multiple myeloma (in Italy since September 2015), it became routinely accessible also to patients with myeloma-associated AL amyloidosis, a particularly fragile population.

Results: We reviewed all SC performed at our center in the last 5 months. Indications for heart uptake were: arrhythmia of unknown etiology. We have correlated the image findings with the clinical and/or pathological diagnosis. AL amyloidosis diagnosis was based on histological demonstration of amyloid by Congo Red staining and immunohistochemistry (IHC) in any tissue and confirmation by proteomics (mass spectrometry (MS)) when indicated.

Results: We performed 704 SC for different indications during the study period. 41/61 patients (67.2%) with AL amyloidosis and cardiac involvement underwent a SC: 27 were negative (86%), 7 (17%) showed some degree of uptake (PS score=2 and focal or univentricular uptake) and 7 (17%) had biventricular uptake (PS score>2). In 3 cases (7%) 5 patients underwent an endomyocardial biopsy (EMB). In 4 cases IHC confirmed AL amyloidosis, while in 1 case IHC was not diagnostic and MS confirmed AL amyloidosis. The two remaining patients were diagnosed of AL amyloidosis by IHC unequivocally in other tissues. Patients with PS 2-3 (73%) 5 patients underwent an endomyocardial biopsy (EMB). Among them, 3 were diagnosed of AL amyloidosis, both by IHC and MS. Two patients, both of them with a M-spike and abnormal free light chains ratio in serum and increased clonal plasma cells in the bone marrow, showed TTR 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 with a negative uptake. 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/or Perugini score 2-3 (PS)) is highly suggestive of TTR amyloidosis. Although some cases have been described with myocardial deposit of both TTR and light chain and up to 10-15% of the population >75 years may show a MC in serum, so it is essential to type accurately amyloid in patients with suspected AL amyloidosis and 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.

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/or Perugini score 2-3 (PS)) is highly suggestive of TTR amyloidosis. Although some cases have been described with myocardial deposit of both TTR and light chain and up to 10-15% of the population >75 years may show a MC in serum, so it is essential to type accurately amyloid in patients with suspected AL amyloidosis and 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.
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 additional 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 GENS01 (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 proteasome inhibitor (PI) and an 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 total of 1073 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 prior refractory status. Clustering of observations at the patient 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 of <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 characters 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 0.28-0.59]).

Summary/Conclusions: Findings from the regression analyses using the updated IMF dataset were consistent with results from the previous analysis3 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:
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 ≥ 4 identify a sub-group of patients with high probability of death within 2 years despite optimized treatment.

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 sub-group of patients with high probability of death within 2 years despite optimized treatment.

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 sub-group of patients with high probability of death within 2 years despite optimized treatment.

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: 3748-3756). 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 MPLlow subpopulation was clearly separated from the SLAMF7low MPLlow population in human CD14+ cells. 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.

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: 3748-3756). 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 MPLlow subpopulation was clearly separated from the SLAMF7low MPLlow population in human CD14+ cells. 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-g 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 monocytic and fibroblastic cells, which express markers of both hematopoietic and stromal cells. Recently, we have reported that clonal neoplastic fibroblasts play a role in the induction of BM fibrosis in primary myelofibrosis (PMF) (Verstovsek, J Exp Med. 2016). We demonstrated that the BM of PMF patients harbors more neoplastic, functionally distinct fibroblasts and fewer MSCs than in normally bone marrow. In addition, we detected an overabundance of fibroblasts 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:** Fibroblasts, which make up <1% of BM cells, differentiate from a subpopulation of CD14+ 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/Scid NO.D-Cg-prkdcscid il2rgtm1wjl/SzJ) with sorted CD14+ monocytes from patients with JAK2V617F-positive PMF or donors with hematologically normal BM engrafted in NSG mice. Transplanted NSG mice with PMF BM-derived CD14+ monocytes developed a myelofibrosis-like phenotype with reticulin fibrosis and abundant neoplastic (JAK2V617F) fibroblasts in the BM and spleen. Two months after transplantation, we detected a subpopulation of hCD45+ and hCD68+ cells within the HLA+ population of BM cells. In addition, we found dysplastic megakaryocytes in the BM and spleen of the PMF CD14+ transplanted mice. Immunohistochemistry staining of paraffin embedded BM sections did not detect hCD3, hCD19 or hCD34 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.

### P691

**ESTABLISHMENT OF AN IN VITRO MODEL FOR THE SKEWED MEGAKARYOPOIESIS BY CALRETTICULIN MUTATION IN HUMAN CELLS**

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**Background:** Somatic mutations on *calreticulin* (CALR) gene are found in a majority of patients with JAK2-mutated or JAK2 wild-type (wt) myeloproliferative neoplasms (MPNs). We and other groups have recently shown that mutant CALR activates the downstream pathway of thrombopoietin (TPO) receptor MPL, which induces factor-independent growth in human and murine cells. However, roles of mutant CALR in human hematopoietic cell differentiation remain largely elusive.

**Aims:** We aimed to recapitulate the MPN phenotypes and examine the impact of CALR ins5 on human hematopoietic cell differentiation in vitro.

**Methods:** We employed IPS cells (iPSC) established from an healthy patient harboring a 5-base insertion mutation in the CALR gene (CALR wt), and CALR gene-knockout (CALR null) Hematopoietic progenitor cells (HPCs) were produced from IPS by “IPS-Sac” method. HPCs were then cultured to induce megakaryocytic cells (MKs) and FLI1 expression for the endothroid cell differentiation in CALR ins5 cells. Finally, we showed that the treatment of ruxolitinib greatly reduced megakaryocytic differentiation in both CALR ins5 and wt HPCs, demonstrating that ruxolitinib does not possess preferential targeting of CALR ins5 cells.

**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.

### P692

**QUANTITATIVE PROTEOME HETEROGENEITY IN MYELOPROLIFERATIVE NEOPLASM SUBTYPES AND ASSOCIATION WITH JAK2 MUTATION STATUS**

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**Background:** Apart from well-known genetic abnormalities, several studies have reported variations in protein expression in Philadelphia negative (Ph−) Myeloproliferative Neoplasm (MPN) patients that could contribute towards their clinical phenotype.

**Aims:** In this context, a quantitative mass spectrometry proteomics protocol was used to identify differences in the granulocyte proteome with the goal to characterize the pathogenic role of aberrant protein expression in MPNs.

**Methods:** LC MS/MS (LTQ Orbitrap) coupled to iTRAQ labeling showed significant and quantitative differences in protein content among various MPN subtypes: Essential thrombocythemia (ET), Primary myelofibrosis (PMF), and according to the genetic status of JAK2 (JAK2V617F presence and 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), shown to be involved in calcium homeostasis and apoptotic signaling, was overexpressed in JAK2V617F 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 ruxolitinib, and could be regulated by JAK2 kinase inhibitors.

**Summary/Conclusions:** In conclusion, these results reveal proteome alterations in MPN granulocytes depending on the phenotype and genotype of patients, highlighting new oncogenic mechanisms associated with JAK2 mutations and overexpression of calreticulin.

### 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**

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**Background:** Systemic mastocytosis (SM) is a myeloid neoplasm defined by abnormal growth and pathologic accumulation of neoplastic mast cells (MC) in various internal organs. The indolent variant of SM (ISM) is associated with an almost normal life expectancy. By contrast, the prognosis in advanced SM, including SM with an associated hematopoietic neoplasm (SM-AHN), aggressive SM (ASM), and MC leukemia (MCL) is poor with short survival times. Most patients with SM express the D816V-mutated variant of JAK2, and could be regulated by JAK2 kinase inhibitors.

**Aims:** The aims of this study were to evaluate the effects of the switch control inhibitor DCC-2618 on proliferation and survival of various neoplastic cells, including mast cells, eosinophils, and monocytes, in patients with systemic mastocytosis (SM).

**Methods:** We employed different human MC lines (HMC-1.1, HMC-1.2, ROSAKITWT, ROSAKITD816V, ROSAKITK509I, MCPV-1.1, MCPV-1.3 and MCPV-1.4) and primary neoplastic MC obtained from patients with SM. In addition, the acute myeloid leukemia (AML) cell lines KLF1 expression required for the endothroid cell differentiation in CALR ins5 cells. Finally, we showed that the treatment of ruxolitinib greatly reduced megakaryocytic differentiation in both CALR ins5 and wt HPCs, demonstrating that ruxolitinib does not possess preferential targeting of CALR ins5 cells.

**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.
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 HMVEC-1 and primary neoplastic cells obtained from patients with AML, chronic myelomonocytic leukemia (CMML) and (clonal or reactive) hyperesinophilia were used. Cell proliferation was quantified by 3H-thymidine uptake. Apoptosis was determined by flow cytometry and light microscopy and phosphoroptokinase-status 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±4 nM) and ROSA KITWT cells (41±6 nM) than in KIT D816V-positive HMC-1.2 cells (123±36 nM), ROSA KITD816V cells (168±65 nM) and the multi-resistant MC line MCPV-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 FM (range: 21-92). Mutations in CALR were predominant in the most prevalent types of AHN 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 anti-apoptotic-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, leukemic 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 (NCT02571036).

P694

DISTRIBUTION OF MUTATIONS IN DRIVER AND NON-DRIVER GENES ACCORDING TO CLONAL HEMATOPOIESIS IN ESSENTIAL THROMBOCYThEMIA AND POLYCYTHEMIA VERA

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Background: Essential thrombocythemia (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 JAK2V617F, 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 18% 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. In 9 patients one driver mutation was detected on SET2 cell line with western blot. To adress the antifibrogenic activity of the drugs and their combinations, we pre-incubated HS27 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).

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 address the antifibrogenic activity of the drugs and their combinations, we pre-incubated HS27 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 was less than 1 (ruxolitinib plus 0.8 µM prednisolone (CI=0.25±0.11) and 32µM ruxolitinib plus 0.8 µM prednisolone (CI=0.45±0.11)). The JAK/STAT signaling pathway was inhibited: the phosphorylation of STAT5 was inhibited by ruxolitinib in 83.2±10.8 % (p-valor<0.05) regarding to control at 30 min and it was maintained at 3 hours (p-valor<0.05). The combinations 32µM ruxolitinib plus 1.6 µM nilotinib (RN) and 32µM ruxolitinib, 1.6µM nilotinib plus 0.8µM prednisolone (RNP) inhibited more than 50% of the phosphorylation of STAT5 at 30 min and maintained at 3 hours. The MAPK signaling pathway was inhibited at 30 min, the phosphorylation of ERK was inhibited in 77.1±16.4%.

Figure 1.
Table 1.

<table>
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<tr>
<th>Sample</th>
<th>Gene</th>
<th>CDS</th>
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<th>% mean CDS</th>
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<th>% indels</th>
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Summary/Conclusions: Gene mutation analysis by targeted NGS in myeloid malignancies is highly reproducible between laboratories and allows a comprehensive characterization of the molecular profile. However, medium-long indels, low frequency mutations (<10%), ASXL1T664fs deletion 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 METHYLMATION 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-0683), 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 at CpG sites regulating chromatin compaction and gene expression/repression. DNAm is known to be altered by ageing and can reflect the effect of diet, lifestyle or disease on cellular processes. Therefore ‘methylation age’ (MA) may be a more accurate reflection of disease than ‘chronological age’ (CA), which is merely a description of how long a person has been alive. Weidner et al (Genome Biology, 2014) described how the measurement of DNAm levels at CpG sites correlates with chronological age (CA) and therefore ‘methylation age’ (MA) may be a more accurate reflection of disease than ‘chronological age’ (CA), which is merely a description of how long a person has been alive. Weidner et al (Genome Biology, 2014) described how the measurement of DNAm levels at CpG sites correlates with chronological age (CA) and therefore ‘methylation age’ (MA) may be a more accurate reflection of disease than ‘chronological age’ (CA), which is merely a description of how long a person has been alive.

Aims: The aim of our study was to correlate MA with disease status, mutational profile and therapeutic response in a cohort of MPN patients treated with Vorinostat.
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 #2007-005360-49). 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 18 Essential Thrombocythaemia (ET) and 22 Polycythaemia 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 -43.4 to +41.6) 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 (-0.8 years vs -15.3, p=0.0122) and after 3 months therapy (-0.5 years vs -12.2, 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).

Suggesions: 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-INNTRINSIC 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 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 induces different malignancies providing a context that favors a certain number 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 mutation.

Methods: To assess the effect of aging on MPN initiation and progression we studied the young and aged inducible transgenic mouse models of MPN. Integrated omics analysis was performed on MPN initiating stem and progenitor cells.

Results: Our results suggest that age related changes in expression patterns result in clones found in aged wildtype mice. The mutated profile 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 HSCPs 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 (hgb <10 g/dL); pts with BL anemia were more likely at BL to have platelet count <50,000/µL (51% vs 38%), pancytopeny MF (71% vs 57%), and high DI PSS 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 BID (12%) arms) (Table). Flow cytometry CAFRBC transfusion independence at 12 weeks (11%) at BL (30% in RUX) in RBC transfusion-dependency was achieved at higher rates with PAC QD (19%) and PAC BID (22%) vs BID (9%); 2 PAC and 0 BID pts achieved RBC-Tb by week 24. In PAC pts, SVR ≥35% and TSS reduction ≥50% were observed regardless of BL anemia or RBC-TD (Table). At BL, 16% of pts in the safety population had grade 3 anemia. Incidence of TE anemia was highest during the first 16 weeks of PAC (20% and 9% weeks 1-8, 13% and 9 weeks 13-18 weeks E and BID, respectively) and first 8 weeks of BID (10%). For pts with BL hgb <10 vs ≥10 g/dL, incidence of grade 3/4 TE anemia was similar with PAC QD (26% vs 28%, respectively), and lower in pts with BL hgb ≥10 g/dL with PAC BID (20%) vs BID (28%). In instances of SD plus clinical benefit, TE anemia with PAC or BID (Table) were in pts with BID hgb <10 g/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 BID. 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.

P700 COMBINATION THERAPY OF POMALIDOMIDE PLUS RUXITINIB IN MYELOFIBROSIS: RESULTS FROM COHORT 1 OF THE MPNSG-0212 TRIAL (NCT01644110)


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Background: Therapeutic options to address anemia in patients (pts) with Myelofibrosis (MF) are limited. In our MPNSG-00109 trial investigating pomalidomide (POM) in MF with cytopenia, anemia was improved in 14-29% of pts treated with 0.5-2.5mg POM once daily (QD) (Schränk FL, Steigmey F et al. Leukemia 2016). Aims: To evaluate synergistic effects of POM plus ruxitinib (RUX), we are currently investigating the combination therapy within the MPNSG-0212 trial (NCT01644110).

Methods: The MPNSG-0212 is designed as multicenter, single-arm phase-Ib/II 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., Blood 2006) and red blood cell (RBC) transfusion independence criteria (Gale et al., Leuk Res 2011). Secondary endpoints are safety, quality of life, progression-free, and overall survival. Main inclusion criterion is MF with anemia (hgb <10 g/dL) and/or RBC transfusion dependency. While POM is given at the fixed dosage of 0.5mg QD, RUX is started at 10mg twice daily (BID) from the first 16 weeks of PAC (20% and 9% weeks 1-8, 13% and 9 weeks 13-18 weeks E and BID, respectively) and first 8 weeks of BID (10%). For pts with BL hgb <10 vs ≥10 g/dL, incidence of grade 3/4 TE anemia was similar with PAC QD (26% vs 28%, respectively), and lower in pts with BL hgb ≥10 g/dL with PAC BID (20%) vs BID (28%). In instances of TE anemia with PAC or BID (Table) were in pts with BID hgb <10 g/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 BID. 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 MF, Cytopenias and Splenomegaly: Results of the 24-week Phase 3 PACRITINIB (MF) and BASAL (BL) THROMBOCYTOPENIA: FOCUS ON RUXITINIB (RUX)-TREATED PATIENTS IN THE PHASE 3 PERSIST-2 TRIAL


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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, JAK1/2 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, IAK1, and CSFIR. 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 RUX due to RUX 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).

Conclusions: This analysis examines outcomes for pts with MF treated with RUX in the phase 3 PERSIST-2 study.
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 (≥5 cm) spleen, were eligible. RUX starting doses were based on baseline platelet count (PLT) (counts (5mg bid [≥50 to <100x109/L], 15mg bid [100 to 200x109/L], or 20mg bid [≥200x109/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 418 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 (100-110 g/L); PLT count, 249x109/L (100-350x109/L); 6.3%). 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 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 asthenia (18.3% [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 ≥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 a ≥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 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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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.
Background: Accurate disease risk stratification is crucial for transplant decision-making in idiopathic myelofibrosis (MF). Despite several prognostic models 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 R² explained variation. All the statistical analyses were performed with IBM SPSS 22.0 and Stata 11.

Results: Median survival from diagnosis in diagnosis of de novo 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 7.44-11.48) 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 R² 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 of the current prognostication systems have a median survival below the 5-year threshold recommended for considering transplantation. Patient selection for transplantation 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), hydroxy-ycarbamide (HC), has mutagenic properties and there is potential for leukemogenicity in patients with this condition. The EXELS study, we report here on 1,590 ET patients from 16 European Centres. Although 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 account for age differences by comparing standard incidence ratios (SIRs) using age-specific cancer registration data.

Aims: To assess the risk of AML and non-hematological malignancies in patients treated with HC or ANA in the EXELS study.

Methods: Previous exposure to ANA and HC was based on patient history. SIRs were calculated using background rates retrieved from Cancer Incidence in Five Continents (CI5). Risk of AML after study enrolment was estimated by cumulative incidence. Minimum exposure time of 180 days was used to account for disease progression as a confounding factor. Informed consent was obtained before the start of the study.

Results: Overall, 3460 patients were exposed to HC, ANA or both at registration, 481 patients had ANA treatment, 2305 had HC treatment and 674 had both exposures to both drugs. The median age in ANA patients was 51 years, and 71 years in HC patients. One hundred and seventy four cases of non-hematologic cancer, including 35 cases of skin cancer, were recorded. SIRs for all malignancies were close to 1 for all treatment groups, indicating similar risks to the background population. For all skin cancers, including melanoma, the SIR for patients with HC treatment was higher than expected, with 20 AML cases observed in the ANA-only group (person-years 8970, SIR 39.7), with another 20 AML cases in the group who switched from HC to ANA (person-years 2934, SIR 91.5). The risk ratio for AML developing in patients who switched from HC more than doubled (RR 2.30-2.52), irrespective of minimum exposure time. In contrast, no AML case was observed in the ANA-only group (person-years 1905, SIR 0) and there were only 3 AML cases in the group who switched from ANA to HC (person-years 802, SIR 68.5). Since the number of AML cases in the ANA group was 0, no statistical comparison could be made. Five AML cases were excluded from analysis since there was uncertainty about which drug was used first.

Table 1. Standardised incidence ratios (SIRs) with 95% confidence intervals (CIs).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Expected</th>
<th>Observed</th>
<th>SIR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>2.30</td>
<td>2.52</td>
<td>0.45</td>
</tr>
<tr>
<td>All cancers</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Skin cancers</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Melanoma</td>
<td>0.22</td>
<td>0.22</td>
<td>1.00</td>
</tr>
<tr>
<td>Other skin cancers</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-Skin Cancers</td>
<td></td>
<td></td>
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<tr>
<td>Leukemia, Lymphoma</td>
<td></td>
<td></td>
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<tr>
<td>Myeloid leukemia</td>
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<tr>
<td>Non-Hematological Malignancy</td>
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</tbody>
</table>

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 INFECTIOUS COMPLICATIONS IN MF PATIENTS RECEIVING RUXOLITINIB. A MULTICENTER STUDY ON 373 PATIENTS
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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.
Background: Infectious complications represent one of 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: At RUX start the clinical features were (median): age 68 years (27-89); ≥65y, 62%; male, 57%; Hb, 10.8g/dL (7-16.7); Hb <10g/dL, 40%; PLT, 246×10^9/L (33-1887); PLT <100×10^9/L, 10%; spleen enlargement, 97%; spleen length ≥10cm, 68%; constitutional symptoms, 52%. International Prognostic Score System (IPSS) was intermediate-1 (15%), intermediate-2 (46%), high (39%). JAKV617F 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, 15% between 6 and 12 months, 9% between 12 and 18 months 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 or somewhat dissatisfied and 14% 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 (18% PV, 27% ET). Overall, 78% of physicians reported that up to 25% of their pts 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 discontinue as key reasons for changing therapy.

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. Interestingly, a proportion 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 ROPEGINTERFERON ALFA-2b-AN UPDATE FROM THE PEGINIVERA 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 institution.
tution. Twenty-nine patients were excluded: 17 for myelodysplastic/myeloproliferative overlap (MDS/MPN), six for insufficient information, five for not meeting criteria for accelerated or blast phase and one patient for a diagnosis of systemic mastocytosis. Of the 158 patients included in the study, the median age at the time of MPN diagnosis and leukemic transformation was 59 and 67 years respectively. Prior MPN diagnosis was: polycythemia vera (PV; n=25, 16%), essential thrombocythemia (ET; n=21, 13%), primary myelofibrosis (n=50, 32%), post ET myelofibrosis (PET MF; n=27, 17%), post PV myelofibrosis (PPV MF; n=24, 15%) and MPN-unclassifiable (n=11, 7%). One hundred and forty-two (93%) patients met the criteria for acute myeloid leukemia, thirteen (8%) had accelerated phase and 3 (2%) patients were diagnosed with myeloid sarcoma. Sixty-four (41%) patients were treated with curative intent including 27 (42%) patients who proceeded to hematopoietic cell transplantation, while 94 (59%) received non curative approach including low dose chemotherapy, hypomethylating agent, clinical trial or best supportive care. The median OS for the entire cohort was 6.5 months (95% CI: 5.06-8.01). In patients treated with curative intent median OS was 8.8 versus 3.2 months (p=0.003) for patients with non-curative intent. There was no difference in OS between historical controls treated between 1998 and 2011 when compared to a more recent cohort of patients (6.5 vs 7.3, p=0.34; see Figure 1). In 105 (67%) patients, NGS molecular profiling of 54 genes (39 hotspot region; 15 complete coding region coverage) was performed on peripheral blood or bone marrow samples using the TruSight Myeloid Sequencing Panel. Mutational data will be correlated with clinical outcomes and insights and clues as to how to develop an individualized treatment approach for this cohort of patients.

Summary/Conclusions: Despite advances in systemic therapies and supportive care, there has been no significant improvement in survival for MPN patients who transform to accelerated or blast phase, confirming that current treatment approaches are ineffective. Results of molecular profiling may provide valuable insights and clues as to how to develop an individualized treatment approach for this cohort of patients.

Other Non-malignant hematopoietic disorders

P709

MASITINIB FOR TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT SYSTEMIC MSTACOTYSIS: 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 WHO classification of Pathology Department, Université Paris Descartes, Paris Sorbonne Cité, Faculté de Médecine & APHP Necker-Enfants Malades, 1Service de médecine interne, Hôpital Tenon, Université Pierre et Marie Curie, Paris, France, 11Département de Pathologie, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, United States, 12Inserm U1068, CRCM (Signaling, Hematopoiesis and Mechanism of Oncogenesis), Inserm U1068, 13CNRS, UMR7258, Institut Paoli-Calmettes, Aix-Marseille Université, Marseille, 14Centre de Référence des Mastocytoses, 15Département of Infectious Diseases and Tropical Medicine and Centre d’Infectologie Necker-Pasteur, Hôpital Necker-Enfants-Malades, Université Paris Descartes, Paris Sorbonne Cité, 16Centre de Référence des Mastocytoses, Université Paris Descartes, Hôpital Necker Enfants Malades, Paris, France

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 16.7% 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 7.6%, 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

M. Machaczka1,2,*, K. Kosior1, E. Pawlowicz2, M. Wolan2, M. Gajewski5, M. Klimkowska3

Background: Hematological malignancies are among the principal etiologies of hemophagocytic lymphohistiocytosis (HLH). The analysis of gene expression profiles in hematological malignancies has provided evidence for a subpopulation of patients with HLH associated with hematological malignancies (M-ALL). This subpopulation is characterized by a poor therapeutic response and increased mortality compared to non-M-ALL HLH. The aim of this study was to report the outcome of patients with M-ALL HLH in a single institution.

Methods: A retrospective analysis of 71 consecutive patients with M-ALL HLH was performed. The patients were treated with a combination of cyclosporine, dexamethasone, and an interleukin-1 receptor antagonist. The response was evaluated based on the International Pediatric HLH-2004 (IPAH-2004) criteria. The outcome was evaluated based on the Kaplan-Meier method.

Results: The median age of the patients was 43 years (range: 18-74). The median duration of therapy was 9 months (range: 1-36). The overall response rate was 75% (53/71). The median survival time was 18 months (range: 2-60). The median duration of remission was 12 months (range: 0-60). The 1-year survival rate was 78% (57/71). The 2-year survival rate was 72% (52/71). The 3-year survival rate was 68% (47/71). The 5-year survival rate was 63% (36/71). The median duration of remission was 12 months (range: 0-60). The median survival time was 18 months (range: 2-60).

Summary/Conclusions: The outcomes of patients with M-ALL HLH treated with a combination of cyclosporine, dexamethasone, and an interleukin-1 receptor antagonist were similar to those reported in other studies. The median duration of remission was 12 months (range: 0-60). The median survival time was 18 months (range: 2-60).

Other Non-malignant hematopoietic disorders
Background: Hemophagocytic lymphohistiocytosis (HLH) is a devastating disorder of uncontrolled immune activation characterized by clinicopathological evidence of extreme inflammation. Hematological malignancy-associated HLH (HM-HLH) has the worst outcome in comparison with any other form of HLH. HM-HLH can occur as the first manifestation of an occult malignancy, before start or during the treatment of known malignancy, or as the sign of a malignancy relapse or transformation to the more aggressive disease form.

Aims: The aim of the present study was to analyze the response to HLH therapy and overall survival of adult patients with HM-HLH.

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, parasitoid, and parasites were also performed if indicated. HLH treatment categories have included proapoptotic chemotherapy (etoposide) and immunosuppressive drugs, targeting hyperactivated macrophages (etoposide, corticosteroids, IVIG) and T cells (corticosteroids, cyclosporine A).

Results: Seventy-one adults, aged 22–84 years, were diagnosed with aggressive HM-HLH during the 8-year period. Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of underlying malignancy, during progression, 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; range 0–2 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±4.13 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 (bacteraemia - 12 patients, viral - 3 patients, fungal - 4 patients; some patients had more than one source of infection). In 2 patients we observed 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 were 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 hematology. HM-HLH 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 HM-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.

P711

WHOLE-EXOME SEQUENCING IN CHILDREN WITH IMMUNE CYTOPEnia: THE APPLICABILITY AND CLINICAL IMPACT

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Background: Whole-exome sequencing (WES) is widely used as a powerful tool for the identification of novel causal variants in primary immune dysregulation syndromes (PIDS). However, the clinical impact of these new causal variants remains uncertain. Our aim was to evaluate the impact and strategy of using WES in children with chronic early-onset autoimmune haemolytic anaemia (AHA), idiopathic thrombocytopenic purpura (ITP) and immune neutropenia, or their combination (Evans syndrome). Most of these patients presented with additional symptoms of immune dysregulation, e.g. common variable immunodeficiency (CVID), lymphoproliferative, autoimmune disorders (diabetes mellitus 1, thyroïditis).

Methods: 30 patients (age 0-39) were evaluated after an examination by a clinical hematologist and signature confirmed consensus. Genomic DNA were prepared from peripheral blood DNA using Agilent SureSelectXT Human All Exon V5/v6/UTR kit and sequenced with the Illumina NextSeq 500 system with a mean coverage of at least 30x.

Results: In 10 patients (33%) we were able to find likely causative mutations. In 3 patients (siblings) we identified a novel variant leading to CTLA4 deficiency, another novel variant in CTLA4 was identified together with an additional pathogenic variant in TSC1, causing a mixed phenotype. The genetic diagnosis of CTLA4 deficiency allowed for the use of CTLA4 agonist (Abatacept) treatment in 1 patient that led to improvement of his symptoms and disease stabilisation. However, after 6 months, the patient had developed agranulocytosis that led to hematopoietic stem cell transplantation. In 1 patient we were able to identify a gain-of-function variant in STAT3 that was recently described in immune dysregulation. In 3 patients we observed variants in genes typically described in connection with antibody deficiency (TACI, CD40L, and lKRBG). In 1 patient with chronic AHA and ITP we found a novel heterozygous variant in TERT gene related to dyskeratosis congenita. In 1 patient with multiple congenital abnormalities and Evans syndrome we discovered a heterozygous variant in KMT2D gene causing Kabuki syndrome. The remainder of our patients harboured variants that posed a diagnostic challenge. In 4 of these we identified variants in genes involved in the pathogenesis of immune dysregulation, which are observed at lower frequencies also in healthy people (CASP10, PIK3CD). 12 patients (36%) had either only one hit in the genes reported causal in autosomal recessive diseases (e.g. ITK, LRBA) or we have not yet found any relevant aberration. In 4 patients we were able to identify novel variants in genes related to immune dysregulation. However, these variants require extensive validation studies, using patients' primary cells or manipulating established in-vitro or animal models with gene editing techniques, in order to prove the causality.

Summary/Conclusions: WES is a highly useful method that helps to identify the genetic cause of the disease in approximately one third of patients and enables targeted therapy. While targeted sequencing can further reduce costs and make analysis more straightforward, gene panels are quickly becoming obsolete as new causal variants are discovered in the rapidly evolving field of primary immunodeficiencies. Because of the heterogeneity of genetic causes of immune cytopenias, we recommend to use WES over targeted gene panel sequencing.


P712

SEQUENCING OF THE HYPOXIA PATHWAY GENES IN PATIENTS WITH CONGENITAL ERYTHROCYTOPATHIES BY NEXT GENERATION SEQUENCING

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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 (Monocytic Erythrocytosis-ME). Aims: Mutation search in the hypoxia pathway.

Methods: We have performed secondary erythrocytosis related to lung, cardiac or renal disorder. Next generation sequencing (NGS) has been used to analyse the presence of mutations in patients suspected of CE.

Results: To date, samples from 140 patients have been recorded, among whom 46 have been analyzed using NGS approach, and 149 harbored the D816V mutation. Of the 14 patients [13 males and 1 female; median age 50 y. (12-71)] with unknown significance were detected, including 4 in PHD genes, 5 in HIF genes, 4 in LNK genes (SH2B3) and 1 in JAK2 gene. 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 JAK2 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 diagnostic 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, exomes including whole exome sequencing are planned to achieve a right diagnosis in the 70% remaining CE patients.

P713 CHARACTERIZATION OF CD34+ HEMATOPOIETIC PRECURSORS IN INDOLENT SYSTEMIC MASTOCYTOSIS AND THEIR POTENTIAL ROLE IN EARLY DISSEMINATION OF THE DISEASE

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Background: Recent studies show that most systemic mastocytosis (SM) patients, including indolent SM (ISM) with (ISMs+) and without skin lesions (ISMs-), 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 MC-committed CD34+ HPC as controls, particularly among ISM cases with MC-restricted KIT mutation (ISMMC); this was associated with progressive blockade of maturation of CD34+ HPC to neutrophil lineage from ISMMC to multilineage KIT-mutated cases (ISMM). 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 ISMsMC and ISMMMC to ISMM patients.

Summary/Conclusions: Positivity for the KIT D816V mutation in PB of ISM is associated with the accumulation of circulating myeloid and monocyte precursors, whereas in BM we observed a different pattern of KIT-mutated cell populations contributing to disease dissemination already at very early stages.
Background: Erythrocytosis, (i.e. increased levels of hemoglobin/hematocrit (Hb/Htc) >95 percentile for age and sex), is rarely found in pediatrics. 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 anecdotal reported.

Aims: We report 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. Erythropoietic Colony Essay (ECE) was performed on peripheral blood with and without cytokines. Clinical features and treatment choices were reported by referring clinicians (table 1).

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>M</td>
<td>Thalassemia</td>
<td>HU, IVIG</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Polycythemia Vera</td>
<td>Hydroxyurea, low dose</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>Essential thrombocythaemia</td>
<td>Aspirin, hydroxyurea</td>
</tr>
</tbody>
</table>

Results: Patients were group 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 Hb positive case was found sporadic. Most Hb variants were not symptomatic, 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 polygenic VHL variant, who presented with arterial hypertension, a small size ganglioencephalocele was found after a 5%rs follow-up. In 21 cases non causes could be identified. They were mostly male (n=18); presented at adolescent age with advanced puberal status (n=17); many were symptomatic (6). Only one 9 year old girl was diagnosed with Polycytemia vera (JAK2V617F positive). Treatment varied according to physician decisions and presence of vascular symptoms, 6 children received ASA and 11 were phlebotomized. In two older year old girl was diagnosed with Polycytemia vera (JAK2V617F positive). Treatment varied according to physician decisions and presence of vascular symptoms. Among the 615 ITP, 181 ES), a significant neurological involvement was observed in 8 patients from 7 centers. With a median (range) follow-up of 12 years (6-26.5), 7 children had ES (including autoimmune neutropenia in 5) and 1 child had isolated ES. Median age at diagnosis was 11.5 years (1.8-15.8). At the last follow-up point, AIC were in partial or complete remission for all patients. Neurological symptoms appeared with a mean delay of 6 years (2.5-18) after AIC onset. The symptomatology was: seizures (n=4), cranial nerve palsy (n=2), Brown-Sequard syndrome (n=2) and/or sensory neuropathy (n=1). No infectious pathogens were identified. MRI showed multiple (n=6) or unique (n=2) inflammatory lesions with hypointense 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 patients. PIDs have been given standard (n=6) or immunomodulatory treatment (n=2), or immunosuppressive treatment (n=3). Cisplatin, Methotrexate and Mofetil, improving symptomatology 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 the 16 patients analyzed had a PID: 22q11.2 microdeletion (n=1), heterozygous C7A mutation (n=2) or homozygous LREA mutation (n=1).

Summary/Conclusions: Neurological involvement is rare and severe late event in the course of childhood ES, or exceptionally AHAi, that may reveal various underlying PID. Complete imaging and pathology examination highlight a causative immune dysregulation and could guide specific therapeutic strategies.

P717

AUTOIMMUNE NEUTROPENIA OF CHILDHOOD SECONDARY TO OTHER AUTOIMMUNE DISORDERS: DATA FROM THE ITALIAN NEUTROPHENIA REGISTRY


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Background: The most frequent Autoimmune Neutropenia (AIN) in childhood is the primary type (p-AIN), whereas in adults AIN is mostly represented by secondary neutropenias, which can be associated to infection, drug adminis-

tration, 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 Onco-

Ematologia 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.

<table>
<thead>
<tr>
<th>Age at onset (years)</th>
<th>Duration (years)</th>
<th>Neutrophil count (x10^9/L)</th>
<th>Anti-neutrophil antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-18</td>
<td>0-24</td>
<td>0.2-1.5</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>19-36</td>
<td>25-36</td>
<td>0.5-2.0</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>&gt;36</td>
<td>&gt;36</td>
<td>&gt;0.5</td>
<td>&gt;1.0</td>
</tr>
</tbody>
</table>

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 chil-

dren (p=0.0009). The median age of onset of AIN was 0.77 year and 10.07 year in p-AIN and s-AIN respectively (p=0.002; p=0.03). The prevalence of IgA deficiency was 4% in p-AIN and 13.6% in s-AIN children: both prevalences were significantly higher than that (0.2%) presented in a group of 470 controls (p=0.0009). The median age 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 lym-

phocytes was significantly reduced (p=0.0001) in s-AIN 1.58 x 10^9/L vs p-

AIN (4.36 x 10^9/L) group. Leucopenia (p=0.0001) occurred more frequently in s-AIN; mononcytosis (p=0.03) and spontaneous remission (p=0.02) in p-AIN. GCSF was used in 9% of the p-AIN and 13% of the s-AIN patients (p=0.004). Neutropenia appeared con-

temporary 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: 566.5 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.

P178 PAROSYMSMAL 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 fre-

quent 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 reg-

istered 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 syn-

drome 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 eculizum-

ab 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 pregn-

ant 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.
Background: Gaucher disease type 1 (GD1), deficient lysosomal acid β-glucosidase 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 recombinant acid β-glucosidase has been the mainstay of therapy for GD1. Eliglustat is an oral substrate reduction therapy approved as first-line treatment for adults with GD1 with poor, intermittent, or extensive CYP2D6-metabolizer phenotypes (>90% of patients). Phase 3 trials demonstrated safety and efficacy of eliglustat 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 5-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 eliglustat tartrate (equivalent to 42 or 84mg eliglustat) 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 the monitoring (plasma levels of eliglustat 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. After 8 years of eliglustat, mean (±SD) hemoglobin level and platelet count increased by 2.1±1.7 g/dL (from 11.3±1.8 to 13.4±2.1 g/dL) and 110% (from 67.5±21.1 to 130.7±59.8 x10^9/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%, CCL-18 by 82%, and glucosylsphingosine (Lyso GL-1) by 88%; 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). Eliglustat was well-tolerated. All quality of life measures (SF-36, fatigue severity score, EORTC core and disease-specific 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 eliglustat, 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.
Aims: The aim of this study was to analyze the biological features of ITP patients refractory to conventional therapies.

Methods: We performed a prospective study in 8 patients with primary ITP not responding to standard therapies (corticosteroids, IVIG and/or splenectomy) as well as in 8 patients with non-refractory ITP (control group). Mean platelet size, surface expression of platelet glycoprotein (GP) IIb and the activation marker CD62 were examined by flow cytometry (FC) analysis, as well as desialylation of platelet membrane receptors using fluorescein-conjugated Ricinus Communis Agglutinin I (RCA-1), a lectin that binds to galactose residues underlying sialic acids. Patients' sera was also incubated with normal human platelets to analyze their ability to induce desialylation in normal platelets. Analysis of desialylation of plasma proteins was performed by Western blot (FXI, FXII) and HPLC (trans-fucose). Patients' sera was also incubated with normal human platelets to analyze their ability to induce desialylation in normal platelets. Analysis of desialylation of plasma proteins was performed by Western blot (FXI, FXII) and HPLC (trans-fucose). Furthermore, TPO-RA refractory patients' sera desilylated normal platelets, but not plasma proteins. MACE assay revealed that unique positivity for anti-GPIbα (TPO-RA) (n=5) displayed higher platelet size and alpha-granule secretion. Recent studies have suggested a mechanism of Fcγ receptors' binding to TPO-RA mediated through receptor internalization and lysosomal digestion. In this study, we aimed to investigate the potential role of anti-GPIbα antibodies in TPO-RA refractoriness.

Results: The characteristics of the patients according to the response to conventional treatments (A: no responders; B: responders) are summarized in Table 1. Non responders exhibited lower platelet counts (p=0.006), higher expression of GPIIb (p=0.049) and loss of platelet sialic acids (p=0.005). Additionally, those who did not respond not only to traditional therapies (corticosteroids, IVIG and splenectomy) but also to thrombopoietin receptor agonists (TPO-RA) displayed higher platelet size and alpha-granule secretion. Furthermore, TPO-RA refractory patients' sera desilylated normal platelets, but not plasma proteins. MACE assay revealed that unique positivity for anti-GPIbα antibodies was only detected in those patients classified as non-responders to conventional ITP therapies, including TPO-RA. 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

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Background: ITP is a disorder characterized by thrombocytopenia resulting from both increased immune-mediated platelet clearance and inappropriate thrombopoietin. 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 increased overall response rates of approximately 80% in poor responders to 1st TPO-RA.

Aims: To present the results of a multicenter survey on TPO-RA switch policies and outcome.

Methods: Charts of ITP pts receiving TPO-RAs at 17 collaborating Haematology Centers were reviewed, including demographics and clinical data. 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 548 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 sequence 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 correlated with lower response rate (p=0.020); escalation of 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=0.066). Adverse events (AE; 16/106 pts) were generally mild and reversible upon discontinuation of either one TPO-RA. One study with thrombopoietin receptor agonists produced 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 their attending physicians to potentially benefit from a switching policy. 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 least likely to respond when switching to the 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 plt fluctuation appears to be linked to the removal of the spleen, the physiological plt reservoir organ.

P723
THROMBOEMBOLIC EVENT MANAGEMENT AND OUTCOMES IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP) DURING TREATMENT WITH ELTROMBOPAG (EPAG): RESULTS FROM THE EXTEND STUDY
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Background: EPAG is an oral thrombopoiesis 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; SAEs; Table 1). We analyzed the characteristics of pts treated with EPAG and any TEE (DVT, n=3; CI, n=3; MI, n=1; other, n=2) 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 responses (target range ≥50–200x10⁹/L). Maintenance dosing continued after minimization of concomitant ITP medication and optimization of EPAG dosing. Pts could remain on EPAG either for 2 yrs in countries where EPAG was commercially available, or for >2 yrs until EPAG became commercially available. The EXTEND primary objective included detection and documentation of AEs, and the EXTEND secondary objective was to evaluate long-term safety and tolerability of EPAG dosing. Pts whose treatment was interrupted 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.

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.

P724
SEVERE BLEEDING IN THE ELDERLY WITH PRIMARY IMMUNE THROMBOCYTOPENIA: CHARACTERISTICS, RESPONSE TO THERAPY AND LONG-TERM OUTCOME
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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 intracranial hemorrhage (ICH) and 74 (14.3%) presented severe (non-ICH) bleeding during ITP. According to multivariate analysis, risk of severe bleeding in patients was increased with platelet count <10x10⁹/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 of pulmonary disease (P=0.001, OR=4.724, 95% CI 1.845-12.092), gum or oral mucosal bleeding (P=0.001, OR=2.941, 95% CI 1.658-5.216) and epistaxis (P=0.027, OR=1.865, 95% CI 1.074-3.238). Compared to severe (non-ICH) bleeding, ICH was more likely incurred in severe bleeding patients with hypertension (P=0.031, OR=2.750, 95% CI 1.286-5.974). Of 103 patients simultaneously observed after diagnosis, 76 (7.7%) patients presented severe (non-ICH) bleeding and 3 (2.9%) patients presented ICH during ITP. Of 222 patients who had bleeding after treatment, 31 patients (13.9%) presented severe (non-ICH) and 4 (1.8%) patients presented ICH. Compared to observation, treatment did not significantly reduce the risk of severe bleeding (χ²=1.889, P=0.169). The total response rate (CR+H) to initial treatment in patients who presented severe bleeding was 58.1% (43/74), which was lower than that in patients without severe bleeding (70.2%, 233/340, χ²=4.014, P=0.045). The response to steroids, IVIG or combination had no significant difference among...
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 <10×10⁹/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

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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), bone marrow endothelial progenitor cells (EPCs) and 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-transplant (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 cultivated 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 cultivated 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 using elevated lectin (sWGA) analyzed by flow cytometry represented the levels of reactive Oxygen Species (ROS) and apoptosis. Proteins expressions for p38, ERK, JNK, Akt were measured 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 the 3 groups. We enrolled three cohorts of subjects increased 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. All patients were randomized into two groups: the CR, R and OR rates were 25%, 41.7% (5/12) and 66.7% (8/12), respectively. In patients who achieved complete response (CR), response(R), and overall response (OR). Secondary end points were complete response (CR), response(R), and overall response (OR). Secondary end points were time to response (TTR) and adverse events.

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Summary/Conclusions: The number and the function of BM EPCs were improved by atorvastatin and NAC treatment with atorvastatin and NAC in vitro and in vivo quantitatively and functionally improved BM EPCs derived from corticosteroid-resistant ITP patients through down-regulation of the p38 MAPK pathway. Although the sample size of clinical study is small, with a relatively short follow-up period by now, our data suggest that atorvastatin and NAC are effective and safe in the management of corticosteroid-resistant ITP patients. Therefore, further prospective multicenter randomized clinical trials with larger sample size are needed in the future.
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-16008542.

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.

Figure 1.

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=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

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 (BCR) 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 ideatisib, 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. A 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: Median improvement in scores from BL were observed early and were sustained through week 48 in VEN treated patients in the EORTC-QLQ-C30 global health status and the role, social, and emotional functioning scales. Furthermore, early and sustained improvements in fatigue through week 48 were seen in both EORTC-QLQ-C30 and EORTC-QLQ-CLL16 (Table 1).

Table 1. (Table of data)

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: This 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 is appealing in 18% of pts, whereas 11% of them believe that a high probability of response upon restarting a TKI. Pts are more likely to stop 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 (28.9%). 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 unadjusted in everyday-life decisions) seemed to be more afraid to lose disease control in CML. ANOVA showed a significant difference (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 authorization to biosimilar rituximab (CT-P10) in all indications of the reference product, including chronic lymphocytic leukaemia (CML). Compared to the originator rituximab, significant price reductions are expected for the reference product offering a more affordable treatment option for CML patients across Europe.

Aims: To assess the budget impact of the introduction of CT-P10 into the treatment of CML in the 28 EU member states. Moreover, we provide an estimation for the number of additional CML 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 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 CML 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 CML patients with CT-P10, a total of 1,624 patients could be treated annually throughout Europe. The potential cost savings in the future 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 five years, 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. Although the UK was the only country where FTF consultations were in place 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.

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 investigate factors influencing their 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 carried out until saturation of themes, transcribed verbatim and thematic analysis was performed using open coding by a doctor, physiotherapist 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 the acceptability of remote monitoring. A screening tool for its identification.

P731
AN INVESTIGATION INTO THE NEEDS AND PRIORITIES OF PATIENTS WITH MULTIPLE MYELOMA DURING REMISSION–IMPLICATIONS FOR RE-DESIGNING PATIENT-CENTRED HEALTHCARE SYSTEMS

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Results: 78 patients were surveyed; the most acceptable alternative was a telephone clinic (with doctor 77%, nurse 69%). 19 interviews were conducted exploring the acceptability of remote monitoring. A screening tool for its identification.

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) study, the addition of the 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. A definitive answer is of a high level of uncertainty and requires examining 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 chemother-apy regimens for adults with ALL in Canada: hyper-CVAD or the Dana Faber 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, overall survival with subsequent event (SAE) and death from causes other than ALL. Event and survival rates were estimated from the GRAALL-R randomized controlled trial by Maury et al. 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-C$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 incremental 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 life years and increased QALYs at a reasonable incremental cost.
avoid reverse causation. 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 studies1,2 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 mainly1,2 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.

Table 1.

| Table: Odds ratios and frequencies of CT scans |
|-----------------|-----------------|-----------------|
| **Cases** | **Controls** | **TOTAL** |
| **OR (95% CI)** |
| CT scans | 0 | 3268 | 3011 |
| 1 | 5 | 12 | 17 |
| 2 or more | 5 | 15 | 20 |
| by type | 1 or more | 9 | 19 |
| | 4.75 (2.53, 15.4) |
| | 1.50 (0.23, 10.5) |
| by age-group | ALL | 6 | 8 |
| | 0.74 (0.46, 1.19) |
| | OTHERS | 3 | 5 |
| | 2.03 (0.28, 15.4) |
| The reference group for all calculated ORs is zero CT scans. |

Methods: We used nationwide, register-based case-control study design to investigate the role of CT imaging in the etiology of childhood leukemia. We identified all childhood (0-15 years) leukemia cases from 1990 to 2011 (N=10953) in Finland and randomly selected thrice as many controls (N=3279) from the Population Registry, individually matched by gender and year of birth. The cases were 81% (N=885) acute lymphoblastic leukemias and 13% (N=142) acute myeloid leukemias. We collected data on all pediatric CT scans from 1975–2011 from the databases of all five university hospitals in Finland and two large central hospitals. In total, we identified 46 CT scans to our subjects. We approximated that this approach covers 81% of all pediatric CT scans performed in Finland from 1975 to 2011. We used a two-year latency period to avoid reverse causation. 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 studies1,2 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
The questionnaire examined patient’s emotions and perceptions during rituximab administration in lymphoproliferative malignancies. Among the 40 patients, 55% were affected by DLBCL and 45% by FL. Over the study period of 2010-2016, we have observed a constant increase in the number of new patients diagnosed with myeloma. The incidence rate in males was 3.8 cases per 100,000 inhabitants/year, compared to 2.2 cases per 100,000 inhabitants/year in females. The incidence of new cases increased from 2010 to 2016, with a peak in 2015 (4.5 cases per 100,000 inhabitants/year) and a decline in 2016 (3.5 cases per 100,000 inhabitants/year).

We retrospectively analysed the incidence of patients with new diagnosed of Multiple Myeloma (NDMM) from 1998 to 2012. We have observed a constant increase in Annual Average of incidence from 4.57 cases/100000 inhabitants/year in the first period to 6.15 in the last. Adjusted by Age Incidence increase from 14 to 18.5 cases in the O65 group. From 2013 to 2016 global and adjusted by Age Incidence remains similar to last years data with 80 new cases and 3.9 cases per 100000 inhabitants/ year from the 1st period to 6.15 in the last. Adjusted by Age Incidence remains similar to last years data with 80 new cases and 3.9 cases per 100000 inhabitants/year. After IMWG 14 criteria to begin treatment in NDMM patients can increment its incidence. New expensive but very effective and well tolerated antilymoma 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 population. 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, 2013-2016). (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 at diagnosis: 74 years (Range: 39-100).

Results: A) INCIDENCE RATES (see Table). In the past IMWG (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 in the first period to 6.15 in the last. Adjusted by Age Incidence increase from 14 to 18.5 cases in the O65 group. From 2013 to 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).

B) PREVALENCE RATES (PreVR).
- 2012. 77 pats alive. PreVR: 22.2 /100000 inhabit;
- 2014. 84 pats alive. PreVR: 24.4/100000 inhabit;
- 2016. 103 pats 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 to 30.3 pats alive /100000 inhabit.) Several new antiMM drugs are available in the therapeutic arsenal and probably increases the prevalence rates.
P738
HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ISOLATED EXTRAMEDULLARY RELAPSE OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

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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 extramedullary sites, mainly the central nervous system (CNS) and the testicles. Although combined therapy is the current treatment for isolated extramedullary relapse (EMR) it is still controversial. To address this issue, we collected data of patients treated with hematopoietic stem cell transplantation (HSCT) for ALL EMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Methods: From 1990 to 2015, 281 children (1-18 years) underwent HSCT for ALL EMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP). Treatment protocols were based on Berlin-Frankfurt-Münster (BFM) Study Group concept, as well as definitions of EMR, CNS relapse, and time of relapse (very early/early/late). HSCT was performed in second complete remission (CR2) or subsequent remission (CR>2), even patients transplanted with active 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 (Tnaplo HSCT).

Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to testes, 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 CR1, 21.0% in CR2 and 6.4% were not in remission Total body irradiation (TBI) was part of 83.6% of conditioning regimens. Overall survival (OS) for the entire cohort was 87% at 3 years and 82% at 5 years. Patients transplanted with active disease (n=43). Patients received largely myeloablative regimens (n=38), or cord blood donor SCT (n=38) in CR1 (n=103), CR2+ (n=105), or CR>2 (n=85), matched- or 1-antigen mismatched unrelated (n=43). Patients received largely myeloablative regimens (n=38), or cord blood donor SCT (n=38) in CR1 (n=103), CR2+ (n=105), or CR>2 (n=85). Twelve percent of patients were transplanted from 1995 to 2000.

Summary/Conclusions: Of the 281 patients treated with hematopoietic stem cell transplantation for isolated extramedullary relapse, the only factors influencing outcome were number of CR and year of transplantation. Patients in CR2 had the better OS (64%), those transplanted with disease had an OS of 44%, those transplanted with active disease had an OS of 38%. TRM for the entire cohort was 10% at 100 days, 11% at 6 months and 1 year and 16% at 10 years, with no difference between HSCT types. Multivariate analysis was conducted after exclusion of patients with active disease at HSCT: the only factors influencing outcome were number of CR and year of transplantation. Patients in CR2+ have a risk of death 2.3 times greater than those in CR2. Children treated after 2000 have half the risk of death then those treated from 1995 to 2000.

P739
PREDICTIVE FACTORS FOR DEVELOPING VENO-OCCCLUSIVE DISEASE IN CONJUNCTION WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN FOLLOWED BY ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Inotuzumab ozogamicin (IO) is a CD22 monoclonal antibody attached to calicheamycin and targets B lymphocytes in early stages of development. In a randomized study of IO compared with conventional salvage therapy in patients with refractory relapsed B-ALL, patients treated with IO had higher complete response rates (81% vs 29%, p<0.001), and a greater proportion of patients proceeded to allogeneic hematopoietic stem cell transplantation (SCT) (41% vs 11%, p<0.001). However, patients treated with IO prior to SCT were also noted to have higher rates of veno-occlusive disease (VOD) compared to the SCT group without IO exposure (11% vs 1%) (Kantarjian NEJM 2016).

In an effort to further investigate this finding, we reviewed transplant outcomes for patients with and without IO exposure.

Methods: We performed a nested control comparison of patients transplanted during the years when they were being treated with IO on a number of clinical trials at our institution.

Results: Between 6/2010 and 10/2016, 251 patients with B-ALL with a median age of 35 years (range, 4-70 years) received an allogeneic matched sibling (n=85), matched- or 1-antigen mismatched unrelated (n=38), or cord blood donor SCT (n=38) in CR1 (n=103), CR2+ (n=105), or with active disease (n=43). Patients received largely myeloablative regimens (busulfan- (64%), fludarabine- (29%) or total body irradiation-based (TBI) (7%). 19% of patients received double allograft regimens consisting of cyclophosphamide-TBI, fludarabine-melphalan-thiotepa, or busulfan-clofarabine-thiotepa. IO was administered to 69 (27%) patients prior to SCT. A median of 3 cycles of IO were administered (range, 1-5 cycles) at a median of 18 days post SCT (range, 6-144 days). Patients were heavily pre-treated, including 18 who had a prior allogeneic SCT. VOD was noted in 21 patients overall (8%) with median onset 19 days following SCT (range, 7-230 days); fatal VOD was noted in 5 patients (2%). VOD was noted in 11 patients treated with IO (16%), and it was fatal in 2 patients (3%). Factors noted to be significant in contributing to VOD in univariate analysis were prior exposure to IO (HR 3.05, 95% C.I. 1.3-7.2, p=0.01) and receiving a busulfan-based transplant preparative regimen (HR 3.4, 95% C.I. 1.02-10, p=0.05); not receiving a prior SCT was significantly protective (HR 0.3, 95% C.I. 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 IO and a double allograft preparative regimen was significantly associated with the risk for developing VOD (HR 5.9, 95% C.I. 1.9-18, p=0.002).

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 allograft preparative regimen increases this risk nearly 6-fold, and should be avoided in these patients.
We report 41 allo-HCTs: 37 from MUD and 4 from MRD performed in 2004-2016. Median age of recipients was 60 (20-71) years and donors 30 (19-53), median time from diagnosis to allo-HCT was 16 (2-307) months. Median size of PNH clone was 80% granulocytes (0.5%>100%). Indication for allo-HCT was PNH with aplastic/hypoplastic bone marrow (19 pts), MDS (2 pts), overlapping MDS/aplasia (3 pts), severe course of PNH with hemolytic crises and transfusion-dependency without access to eculizumab (17 pts). Additional risk factors were Budd-Chiari syndrome and hepatosplenomegaly (1 pt), history of renal insufficiency requiring hemodialysis (2 pts), chronic hepatitis B (1 pt) and C (1 pt). The preparative regimen consisted of treosulfan 3x14 g/m² plus fludarabine 5x30mg/m² (31 pts) or treosulfan 4x14 g/m² plus cyclophosphamide 4x40mg/kg (10 pts). GVHD prophylaxis consisted of cyclosporine-A, methotrexate and pre-transplant ATG in MUD-HCT. 2 pts instead of cyclosporine-A received mycophenolate mofetil and tacrolimus. Source of cells was bone marrow (13 pts) or peripheral blood (28 pts) with median 6.3x10^10NC/kg, 7.5x10^6CD34+cells/kg, 24.7x10^6D3+cells/kg and a median number of 3000-7000 cells/µl. TRAEs for these subgroups were similar to the overall study results. Factors contributing to survival in these pts is a potential area for future exploration.

Support: Jazz Pharmaceuticals.

P742
A COMPARISON OF CLINICAL OUTCOMES BETWEEN MATCHED SIBLING DONOR (MSD) AND UNRELATED DONOR (URD) STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH SEVERE APLASTIC ANEMIA
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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), respectively.

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 MSD group was significantly shorter compared to those of the WM-URD and the PM-URD groups across all subgroups (13 days, 16 days, and 16 days; P<0.01). The incidence of acute and chronic GVHD of the WM-URD and the 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 44.6% and 33.3% vs 8.9%; P<0.01, respectively). When we compared the incidence of transplant-related mortality (TRM; 10.7% vs 7% and 6%; P=0.53) and overall survival rate (OS; 89% vs 92% at 6 years; P=0.52) between the WM-URD and the MSD groups, there were no significant difference. However, trends of higher TRM incidence (18.2% vs 7% at 4 years; P=0.05) and lower OS rate (81.8% vs 92.5% at 6 years; P=0.05) were observed between the PM-URD and the MSD groups. There was no primary graft failure in any group. Post-HSCT graft failure of both WM-URD (0% vs 18.3%; P=0.01) and PM-URD (0% vs 18.3%; P=0.02) groups were significantly lower compared to that of the MSD group. When we adjusted other clinical and transplant-related factors, which include age and IST failure, using multivariate

Summary/Conclusions: In this study, diagnostic criteria requiring onset by day 21 would exclude >26% of pts with VOD/SOS, with more than a third of these being pediatric pts. This highlights the importance of including late-onset VOD/SOS in diagnostic criteria. With DF, 52.8% were estimated to survive to Day +100 (60.4% of pediatric and 48.7% of adult pts). TRAEs for these subgroups were similar to the overall study results. Factors contributing to survival in these pts is a potential area for future exploration.

Support: Jazz Pharmaceuticals.
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 THALASSEMSIA 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/d 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) 50mg/kg on days SCT -12,-11,-10. Flu 35mg/m2 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 chimera. 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, alloSCT) 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 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 Thiotepa (10mg/kg). Here we compared outcomes of 45 such patients (getting augmented regimen, Flu-Bu with the addition of Thiotepa, (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 Thiotepa (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 Thiotepa 5mg/kg for 2 days (10mg/kg). The conventional myeloablative regimen was identical, however without the addition of Thiotepa.

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 venoocclusive disease in 2% of group 1 and 4% of group 2, (p=1.0) and comparable non- relapse mortality (NRM). 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.

Figure 1.
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.

P745
PROGNOSTIC TOOLS CAN PROVIDE PERSONALIZED OUTCOMES PREDICTION AFTER ALLOGENEIC HCT IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES
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Background: Current prognostic indices for allogeneic HCT (alloHCT) outcome often focus on a limited set of factors, be they patient characteristics, disease features, or transplant approaches. We sought to evaluate two comprehensive prognostic models in a large sample of patients undergoing alloHCT with CD34 selection (CD34 alloHCT).

Aims: To evaluate two comprehensive prognostic models: The first combining the HCT-Comorbidity Index (HCT-CI) and Disease Risk Index (DRI); the second applying the Center for International Blood and Marrow Transplant Research (CIBMTR) One Year Survival Outcomes Calculator, which uses large-scale multicenter data reported to the CIBMTR to provide patient-specific predictions on survival 1 year after first alloHCT.

Methods: This retrospective analysis included adult recipients of first alloHCT with CD34+ selected PBSCs from 7/8 or 8/8 donors for AML, ALL, or MDS at a single center between 1/2000 and 12/2015. The Kaplan-Meier (KM) method estimated OS and RFS. The cumulative incidence method for competing risks estimated relapse and nonrelapse mortality. We evaluated univariate association between variables of interest and OS/RFS using the log-rank test. Cox regression models assessed the adjusted effect of covariates on OS/RFS. We then determined predicted 1 year OS for each patient using the CIBMTR Calculator. Patients were divided into groups based on predicted OS probability.
Aims: We had generated two composite endpoints: in both III-IV acute GVHD (aGVHD), relapse, death or chronic GVHD (cGVHD) requiring systemic treatment. In 2016 EBMT annual meeting a redefinition of this endpoint was proposed changing cGVHD event from those patients with cGVHD requiring systemic treatment (the original one) to those with just severe cGVHD (the redefined one).

Table 1.

Background: Disease free survival is the most used common endpoint for clinical research on allogeneic stem cell transplantation (HSCT), but it doesn’t include morbidity endpoints or those which affect their quality of life as graft failure (GF), disease relapse, or death. Furthermore, this endpoint may overestimate the benefit of new treatments if baseline differences exist. All-cause mortality (ACM) and disease free survival (DFS) are composite endpoints, which are useful for comparing outcomes of alloSCT to those of non-myeloablative and matched-related donor alloSCT.[1] However, ACM may not elicit the timing of benefit as early as DFS. Cumulative incidence of TMA was 3.4% (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 CML/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 immunoprophylaxis.

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.

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Background: Disease free survival is the most used common endpoint for clinical research on allogeneic stem cell transplantation (HSCT), but it doesn’t include morbidity endpoints or those which affect their quality of life as graft failure (GF), disease relapse, or death. Furthermore, this endpoint may overestimate the benefit of new treatments if baseline differences exist. All-cause mortality (ACM) and disease free survival (DFS) are composite endpoints, which are useful for comparing outcomes of alloSCT to those of non-myeloablative and matched-related donor alloSCT.[1] However, ACM may not elicit the timing of benefit as early as DFS. Cumulative incidence of TMA was 3.4% (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 CML/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 immunoprophylaxis.

P746

THROMBOTIC MICROANGIOPATHY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: IS THERE A PROTECTIVE ROLE FOR URSOODEOXYCHOLIC ACID?

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’s incidence was: 90 (15%) for III-IV aGVHD, 170 (27%) for cGVHD, 152 (25%) for relapse and 57 (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; C195% 0.53-0.95), >4 prior lines (p=0.03, HR 1.5, C195% 1.04-2.04), early EBMT stage (p<0.001 with early as reference; intermediate p=0.002, HR 1.5, C195% 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 the morbidity is misdiagnosed. The EBMT disease score was the factor with more impact in both; it is interesting to point that although the group is smaller, 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% for 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 final 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, (29% patients whom had MOD) and 430 patients (43.0%) ≥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 58.9% (95% confidence interval [CI], 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, 210 patients (21.0%) had ≥1 treatment-related adverse event (TRAE). TRAEs occurring in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypertension (2.0%).

Figure 1.

Summary/Conclusions: This final analysis of the defibrotide expanded-access protocol demonstrates favorable Day +100 survival (58.9%) in patients with confirmed VOD/SOS following HSCT, and 49.5% in those with MOD, a complication typically associated with dismal outcomes. Survival and safety findings, consistent with prior clinical trials, provide supportive evidence for the clinical utility of defibrotide for treatment of VOD/SOS in patients with and without MOD. Support: Jazz Pharmaceuticals.
Stem cell transplantation - Experimental

**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 LSK (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 LIM-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 T and B cells. HPC(LSK) cells are CAR-T cell targets since they are established from a range of transgenic mice, underlying the overall applicability of this model. Using this system, we established LSC lines that express BCR/ABL210, MLL-AF9,Nras12/12 or Flt3/ITD; Nras12/12. These LSCs home to the bone marrow, differentiate into all lineages and drive myeloid leukemia in mice.

**Summary:** We created the most powerful method of expanding hematopoietic stem/progenitor cells. They are immortalized and can be expanded indefinitely. This allows the analysis of 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.

**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 donor immunity. The dual challenge of alloHSCT is therefore to 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-transplantation will allow reduced intensity conditioning (RIC) and promote both donor T cell engraftment and GVL whilst reducing the risks of GVHD.

**Methods:** We used a MHC-mismatched mouse model of alloHSCT, where donor T and NK cells are hematopoietic cell pools. HPC(LSK) cells were injected into NSG recipients that were established from a MHC-mismatched mouse model of alloHSCT.

**Results:** We extended our observations in BCL2 in WT mice with just two doses of ABT-199 resulted in rapid depletion of NK cells. Our preliminary data indicates that alloHSCT WT recipient mice pre-treated with ABT-199 develop full donor engraftment even in the setting of significant RIC, with minimal GVHD.

**Summary/Conclusions:** Recipient NK cell inhibition may therefore represent a means by which to deliver alloHSCT more safely by reducing conditioning intensity and GVHD.

**P751**

**MESENCHYMAL STEM CELL IRRADIATION INTERFERES WITH THE ADIPOGENIC/OSTEOGENIC DIFFERENTIATION BALANCE IMPROVING THEIR HEMATOPOIETIC-SUPPORTING ABILITY**

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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:** Seven BM samples were obtained from healthy donors after informed consent. MSC were obtained and characterized following standard procedures of MSC isolation and culture. Then, one aliquot was gamma-irradiated with a single dose of 2.5 Gy 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 annexin/V/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 former experiments, BM specimens, CD34+ cells were isolated from leukaemopheresis 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, respectively). In addition, angiopoietin 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.

**Summary/Conclusions:** Irradiation of MSC with 2.5 Gy improves their hematopoietic supporting ability and modifies their differentiation capacity, increasing the osteogenesis and decreasing the adipogenesis.

Funding: PI12/01775 (ISCIII, Spain). Santander-USAL grant to SP.

**P752**

**DYSFUNCTION OF BONE MARROW MESENCHYMAL STEM CELLS FROM PATIENTS WITH PROLONGED ISOLATED THROMBOCYTOPENIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION CAN BE IMPROVED BY N-ACETYL-L-CYSTEINE**

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P754

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 T 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 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-2bxd) or in B6.bm12 mice (CD45.2, H-2b). Th9 cells were induced in vitro from CD326+ intestinal epithelial cells from KO and WT animals after allo-BMT. The cIAP-/-and -XIAP-/-animals showed increased number of Th9 cells and significantly reduced expression of anti-apoptotic protein Bcl-2 and LC-3-3 that contribute to the expression IL-9 from B clonally B-GVHD animals was significantly increased.

Summary/Conclusions: These data suggest that enhanced apoptosis in the target tissues in the absence of IAPs contributes to greater GVHD severity. Thus expression of functional IAPs in target tissue is crucial for reducing the damage from GVHD.
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-g-, 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-, IFN-g- and anti-IL-12 phenotype and instead expressed TNF-a and IFN-g without strong systemic increase in these cytokines. Since TNF-a and IFN-g 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.

**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-a and IFN-g without strong systemic increase in these cytokines. Since TNF-a and IFN-g 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.

**P755**

**IMPROVED HSC ENGRAFTMENT IN A MOUSE MODEL OF HEMATOPOIETIC STEM CELL GENE THERAPY MEDIATED BY MSCS**

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**Background:** Co-transplantation of human mesenchymal stromal cells (hMSC) has been reported to reduce the risk of graft failure and improve hematopoietic stem cell (HSC) engraftment in xenogeneic and determined allogeneic transplantation. In addition, we have demonstrated that the co-infusion of MSCs with low numbers of purified HSCs significantly improves short- and long-term hematopoietic reconstitution in an autologous HCT experimental model with sunitibdelating (5Gy).

**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 1,500-3,000 Fanca-/- LSK cells transduced with a therapeutic lentivector (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-correced LSK were infused. Once again, Ad-MSCs co-infusion increased 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.

**P756**

**EFFECT OF POMALIDOMIDE ON T CELL POLARIZATION IS MEDIATED THROUGH EPIGENETIC MODIFICATIONS.**

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**Background:** There is conflicting evidence regarding the potential use of IMiDs and particularly pomalidomide after allogeneic stem cell transplantation (allo-HSCT). It has been shown that these drugs downregulate Th1 phenotype increasing IFN-g cytokine production via the augmentation of T-bet transcription factor. This effect might increase the risk of GVHD after allo-HSCT. Nevertheless, a recent trial has reported a potential benefit of the use of pomalidomide as GVHD treatment.

**Aims:** In the current study, we have analyzed the effect of pomalidomide in the polarization of CD45RA+ cells and the epigenetic mechanisms that might be involved in this effect.

**Methods:** Isolated CD45RA+ T cells from healthy donor’s Buffy Coats were treated with anti-CD3 plus anti-CD28 and supplemented with IL-12, IFN-g and anti-IL-12 for 5 days. Pomalidomide at two different doses (10 and 100 nM) were added into the culture and the effect on T cells polarization was analyzed by flow cytometry after staining with anti-CD25, anti-IFN-g, anti-CD4 and anti-IL-2 for Th1 cell polarization and anti-CD25, anti-IL-10, anti-CD3 and anti-IL-4 for Th2 cell polarization. In addition, the release of cytokines (IL-2, IL-4, IL-6, IL-10, TNF-a and IFN-g) in cell culture supernatants were measured by BD Human Th1/Th2 Cytokine BCA kit (BD Biosciences) and T-bet and GATA-3 expression were analyzed by Western Blot. Chromatin immunoprecipitation (ChIP) assays were performed to assess the trimethylation of H3K4 (associated with gene activation) and the trimethylation of H3K27 (associated with gene repression) in the TBPET and GATA-3 gene promoters.

**Results:** Pomalidomide increased the expression of IFN-g and IL-2 as determined by flow cytometry in Th1 cell culture conditions. By contrast, in the presence of IL-10, IL-4 and IL-12 we observed a strong decrease in IL-10 and IL-4 upon adding pomalidomide to the culture. In addition, the exposure to pomalidomide increased the levels of TNF-a, IFN-g and IL-2 in the Th1 polarizing culture while, under Th2 promoting conditions, an increased concentration of IL-4 and IL-2 in supernatant was observed after exposure to pomalidomide. Furthermore, exposure to pomalidomide led to an increased expression of T-bet as assessed by western-blot in naive CD45RA+ cells activated with anti-CD3 plus anti-CD28 and supplemented with IL-12, IFN-g and anti-IL-4. By contrast, in Th2 polarization conditions, pomalidomide increased GATA-3 expression. We next studied whether or not the effect of pomalidomide in T cell polarization might be mediated by epigenetic mechanisms: in the presence of Th1 promoting conditions there was a significant increase of the activation marker H3K4me3 at the TBPET promoter and a significant decrease in H3K27me3 upon exposure to the drug while, under Th2 promoting conditions, a significant increase in H3K4me3 at the promoter of GATA-3 gene was observed among the cells exposed to pomalidomide.

**Summary/Conclusions:** Pomalidomide favours both Th1 and Th2 cell differ-entiation of CD45RA+ cells depending on the cytokines present in the medium. Treatment of naive T cells with pomalidomide induces epigenetic modifications during T cell polarization which might favour the process of differentiation of the naive T cells.

**P757**

**MESENCHYMAL STEM CELL (MSCS) ATTENUATE CUTANEOUS SCLERODERMATOUS GRAFT-VERSUS-HOST DISEASE (SCL-GVHD) THROUGH INHIBITION OF IMMUNE CELL INFILTRATION IN A MOUSE MODEL.**

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**Background:** Human chronic graft-versus-host disease (CGVHD) shares clinical characteristics with a murine sclerodermatous GVHD (Scl-GVHD) model that is characterized by skin thickening and lung fibrosis.

**Aims:** This study investigated the therapeutic effect of mesenchymal stem cells (MSCs) on the development of Scl-GVHD according to each target organ.

**Methods:** A B10.D2 → BALB/c transplant model of Scl-GVHD was used to address the therapeutic effect of mesenchymal stem cells (MSCs) on the develop-ment of CGVHD. M210B4 cells were administered after allo-HSCT at a dose of 3 x 10^7 cells/mouse on days 3, 5, 7.

**Results:** The clinical and pathological severity of cutaneous Scl-GVHD was significantly attenuated in MSC-treated recipients relative to Scl-GVHD controls. After MSC treatment, skin collagen production was significantly reduced with consistent downregulation of TGF-β expression. Effects of MSCs on molecular markers implicated in persistent TGF-β signaling and fibrosis, such as phosphatase and tensin homolog (PTEN), phosphorylated Smad-2/3 and matrix metalloproteinase-1 (MMP-1), were observed in skin tissue. MScs neither migrate to the skin nor affect the in vivo expansion of immune effector cells, but inhibited their infiltration into skin via downregulation of CCR4 and CCR8 expression. Furthermore, when cultured on GM-CSF+ T cells, CD45RA+ T cells and CD45RA+ CD11b+ monocyte/macrophages. MSCs diminished expression of chemokines such as CCL1, CCL3, CCL8, CCL17, and CCL22 in skin. MSCs were also dependent on stimulated spleno-cyes to suppress fibroblast proliferation.

**Summary/Conclusions:** Our findings indicate that MSCs attenuate the cuta-neous Scl-GVHD by selectively blocking immune cell migration and downreg-ulating chemokines and chemokine receptors.

**P758**

**C57BL/6 SUBSTRAINS SHOW DIFFERENCES IN HEMATOPOIETIC REPOPULATION AND ONCOGENICITY.**

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**Background:** C57BL/6 mice are one of the most studied in-bred mouse strains.
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 Nnt gene in C57Bl/6J mice sensitizes HSPCs to oxidative stress, which compromises their self-renewal, differentiation, and survival. The absence of a functional Nnt gene in J-HSC may curtail their ability to resolve elevated 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+cKit+ 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. The lineage potential and repopulating activity of multi-potent progenitors (MPP2: Lin-Sca1+cKit+Flt3+CD48+CD150+, MPP3: Lin-Sca1+cKit+Flt3+CD48+CD150-, MPP4: Lin-Sca1+cKit+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 vitro 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 HSC 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 J mouse compared to 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 J mouse compared to 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.

**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 were: 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 (IRF), platelet count (PCT), total iron binding capacity (TIBC), saturation index (SI), serum ferritin (Fe), total iron binding capacity (TIBC), and VTE risk. These data could be useful to investigate genes related to VTE.

**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 VTE 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 allowed us to correlate genetic correlation of VTE 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>HI</td>
<td>rs5810971</td>
<td>11</td>
<td>Cis</td>
<td>PRKASX1GAP</td>
<td>1.00-6</td>
</tr>
<tr>
<td>RDW</td>
<td>rs2855336</td>
<td>12</td>
<td>LSS</td>
<td>SCRT3</td>
<td>3.17-6</td>
</tr>
<tr>
<td>BF</td>
<td>rs5672284</td>
<td>15</td>
<td>4/2</td>
<td>TICAM2</td>
<td>2.27-6</td>
</tr>
<tr>
<td>SAT</td>
<td>rs919799</td>
<td>17</td>
<td>2/2</td>
<td>CUBN5</td>
<td>3.17-6</td>
</tr>
<tr>
<td>TFR</td>
<td>rs2519456</td>
<td>17</td>
<td>InS</td>
<td>GNTT1</td>
<td>3.17-6</td>
</tr>
</tbody>
</table>

G: genotypic correlation with VTE; Chr: Chromosome.

**Summary/Conclusions:** Several genetic variants involved in the variance of erythrocyte phenotype levels were identified by GWAS. Of note, TFR was associated with a SNP in TFPI2 that might influence the variance of both TFR levels and VTE risk. These data could be useful to investigate genes related to red blood cell parameters and VTE.

**Reference**


This work was supported by RIC RD12/00420032, FIS PI12/00612 and FIS PI 15/0269 grants

**P760**

**ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) PATIENTS SHOW AN INCREASED THROMBUS FORMATION IN A DYNAMIC MODEL OF PLATELET ADHERENCE**

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1Madrid, Spain, June 22 – 25, 2017

haematologica | 2017; 102(s2) | 307
Aims: We wanted to evaluate, in a group of ET and PV patients, the PLT thrombus formation capacity by an ex-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 38-87) were enrolled after informed consent. For the adhesion assay, peripheral venous whole blood was drawn in sodium citrate, recalcified 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) 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 P-selectin or phosphatidylserine. 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±1.7%), while no difference was found between ET and PV patients. The analysis according to 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=NS. vs JAK2-V617F), while, PLT adhesion of MPL-positive (n=3; coverage: 32±1.2%) or triple negative (n=13; coverage: 42±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 subjects on aspirin or hydroxyurea or placebo. PLT count correlated (p<0.01) with PLT adhesion only in CalR-positive ET patients. The analysis of adherent PLT surface markers shows no difference in P-selectin expression between whole patients and controls. Differently, phosphatidylserine expression was significantly reduced (p<0.01) in both ET and PV compared to healthy subjects.

Summary/Conclusions: ET and PV/platlets show an increased PLT thrombus formation potential, particularly in patients carrying the JAK2-V617F mutations. 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. 14505 of the “Italian Association for Cancer Research” (A.I.R.C.)].

P762

Abstract withdrawn.

P763

INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS UNDERGOING LOWER LIMB SURGICAL REVASCULARIZATION: IS THROMBOPROPHYLAXIS WARRANTED?

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3Copenhagen, Denmark
4Department of Internal Medicine, CARIM, Maastricht University, Maastricht, Netherlands

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>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balloon angioplasty</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Bypass aorta-femoral</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Bypass femoral-peroneal</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Bypass femoral-femoral</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Bypass femoral-polipetal</td>
<td>252</td>
<td>2</td>
</tr>
<tr>
<td>Bypass femoral-tibial</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Lower limb embolectomy</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Femoral artery exploration</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Thrombectomy</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>354</td>
<td>5</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, I2=0.048, p<0.001). 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), requires 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 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>h2</th>
<th>p (value)</th>
<th>Covariance</th>
<th>Age, smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12 (nmol/L)</td>
<td>44.1±40.7 (74.4558)</td>
<td>0.67</td>
<td>2.95x10^-17</td>
<td>0.11</td>
<td>Age, smoking</td>
</tr>
<tr>
<td>SF (nmol/L)</td>
<td>2.1±0.6 (0.2-4.1)</td>
<td>0.27</td>
<td>2.3x10^-6</td>
<td>0.07</td>
<td>Age, smoking</td>
</tr>
<tr>
<td>RCF (nmol/L)</td>
<td>124.0±63.7 (47.5354)</td>
<td>0.62</td>
<td>1.85x10^-1</td>
<td>0.36</td>
<td>Age, smoking</td>
</tr>
<tr>
<td>HCY (nmol/L)</td>
<td>8.4±4.5 (2.7-9.77)</td>
<td>0.36</td>
<td>3.61x10^-1</td>
<td>0.41</td>
<td>Age, 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 variants</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>6</td>
<td>MTFR914033</td>
<td>2.1x10^-6</td>
</tr>
<tr>
<td>RCF</td>
<td>11</td>
<td>TMFR914033</td>
<td>4.7x10^-15</td>
</tr>
<tr>
<td>HCY</td>
<td>9</td>
<td>INRFR914033</td>
<td>1.3x10^-7</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 (CalR), thrombopoietin receptor (MPL)] and concomitant cytoreductive or antiplatelet therapies was also evaluated.

Methods: Thirty-seven ET (19 JAK2V617F, 9 CalR 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 (leukocyte marker), and anti-CD235 (erythrocyte marker) monoclonal antibodies were used. Annexin V (AnnV) staining was used to evaluate the expression of procoagulant phosphatidylycerine 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 CalR 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 patient's presenting different mutations. Importantly, the lack of correlation found between the total and subtype-specific MP counts with the corresponding cell of origin counts suggests an active stimulation of MP formation.

Project funded by AIRC-IG2013 N.14505 of the Italian Association for Cancer Research (AIRC).

P767
ARE WE TESTING APPROPRIATELY FOR THE LUPUS ANTICOAGULANT (LA)?
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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 fulfilled 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 (31%), rheumatology (17%) and clinical haematology (11%). 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-beta2-glycoprotein I as well as the lupus anticoagulant.

P768
RESULTS OF USING BRIDGING THERAPY WITH SODIUM BEMIPARIN AT THERAPEUTIC-DOSE
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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 (UFH) in the treatment 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/Ia ratio and average life. The sodium bemiparin is the LMWH with greater anti-Xa/Ia 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 safety (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 CHADS/VASC ≥2, aged between 15 and 92, with an average age of 69 years old. The reasons of anticoagulation in our patients were atrial fibrillation, 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 of a prophylactic dose (warfarin) before the procedure, and replacing it by sodium bemiparin at full doses <50 kgr: 5.000 IU/24h, 50 to 70 kgr: 7.500 IU/24 h, 70-100 kgr: 10.000 IU/24 h and >100 kgr: 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, valvular replacements etc), 340 cases of minor surgery (removal of neus, 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 AVK (epistaxis, petechiae and bruises, hemoptysis, menorrhagia and gastrointestinal bleeding), 30 cases of hemorrhagic complications (major and minor bleeding) resulting from this use.

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.
IBRUTINIB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA: 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 offers a treatment without chemotherapy. The phase 3 RESONATE trial 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 weekly 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. OS is defined as the time from randomization to death or last contact.

Results: Randomized 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. The 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, AEs for the del11q subgroup tended 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). Adverse events (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 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 8%; pneumonia: 11% vs 4%; atrial fibrillation: 4% vs 2%, 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.
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 7%. As determined by an independent review committee at the initial assessment was performed beginning with the first clinical assessment of CR or PR with nodes <2 cm and then every 12 weeks until MRD negativity (defined at 10^-4 sensitivity). MRD was assessed by NGS and multicolor flow cytometry and the best response was reported. Data cutoff date was June 10, 2016.

Results: Pts (N=158) had a median age of 67 years (range, 29–85); a median of 4 (range 0–10) 32% were fludarabine refractory; 11% previously had been treated with B-cell receptor signaling inhibitors (BCR); 48% had nodes ≤5 cm; and 78% had unmutated IGHV. The median duration of venetoclax therapy was 16.7 months (range 0–34.4 months). Primary reasons for discontinuation (50.6% of pts) were PD (31.0%), adverse events (AEs) (12.6%), withdrawal of consent (2.5%), stem cell transplant (2.5%) and other (1.9%). For all 158 pts, the investigator-assessed ORR was 77% and CR rate was 18%. The 24-month estimates for progression-free survival (PFS) and overall survival (OS) were 52% and 72%, respectively. The safety expansion cohort included 5 pts with previously untreated del(17p) CLL. These pts had an ORR of 80%, CR rate of 40%, and all 5 were alive and progression-free 1 year after their first treatment administration. Among the 18 pts with prior BCR therapy, ORR was 61% and CR was 11%, with 12-month PFS and OS rates of 50% and 72%, respectively. The most commonly reported AEs were neutropenia (42%), nausea (37%), diarhea (37%), anemia (24%), and fatigue (22%). The most common grade 3–4 AEs were neutropenia (39%), thrombocytopenia (15%), and anemia (14%). Infection rate (77% all grades, 22% grade 3–4) and spectrum were consistent with the underlying disease. The rate of laboratory tumor lysis syndrome (TLS) was 5%, with no cases of clinical TLS. Of 101 pts with evaluable blood MRD by flow cytometry, 76 also had MRD data by NGS. From the full trial cohort of 158 pts, 42 (27%) demonstrated blood MRD negativity at 10^-4 by flow cytometry, and 28 had a contemporaneous NGS sample. MRD negativity (10^-4 sensitivity) was confirmed by NGS in 20 pts (71%), and 8 pts (29%) were MRD-positive by NGS. MRD negativity by NGS (10^-5) was observed in 22 pts in blood and bone marrow samples, and 15 pts were negative in the marrow although not necessarily at the same time. One pt negative by NGS did not have a matching flow cytometry assessment and 1 pt was positive by flow cytometry (0.008% vs 0.02%). Pts who achieved blood MRD-negative CR by flow cytometry (n=19) had a 24-month PFS estimate of 100%, compared with 78.5% for pts who had blood MRD-negative CR by flow cytometry. These results were obtained when assessed with a 10^-4 cutoff.

Summary/Conclusions: Venetoclax monotherapy resulted in a high response rate that was durable in this high-risk population, including among pts who had previously received a B-cell receptor inhibitor. MRD negativity by either flow cytometry or NGS correlated with outstanding outcomes.

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 and JAK selective inhibitors synergize to suppress this response (Coffey et al., 2013). Hence, BCR/SYK and cytokine JAK/STAT signaling pathways cooperate to control B cell activation. This cooperation appears to be relevant to B cell malignancies as well. IL-4 in normal B cells synergizes to suppress this response (Coffey et al., 2013). Hence, BCR/SYK and cytokine JAK/STAT signaling pathways cooperate to control B cell activation. This cooperation appears to be relevant to B cell malignancies as well. IL-4 in normal B cells synergizes to suppress this response (Coffey et al., 2013). Hence, BCR/SYK and cytokine JAK/STAT signaling pathways cooperate to control B cell activation. This cooperation appears to be relevant to B cell malignancies as well.

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), ibritinib dosed at 420mg (CLL) or 560mg (NHL), both on C1D1.

Results: 38 pts were enrolled: 20 CLL/SLL and 18 NHL, including 6 follicular (FL), 6 DLBCL, 4 mantle cell (MCL) and 2 marginal zone (MZL). Med age 65 yrs (range 32-85); 29 M/9 F; med prior tx=3 (range 0-6). 2 pts ref to prior PI3Kδ/BTK prev treated with ibritinib (1 rel/ref rel). MTD was not reached. Most common (>20%) all causality AEs were fatigue (42%), diarhea (39%), dizziness (34%), nausea (32%), neutropenia, pyrexia, rash, infusion reaction, insomnia (each at 29%), thrombocytopenia, cough (each at 26%), anemia (24%) and sinusitis (21%). ORR 34% (all AEs were minimal), the only event >10% was neutropenia (16%). ORR amongst 36 evaluable pts is shown in the following Table 1.

Table 1.

S773
THE DUAL SYK/JAK INHIBITOR CERDULATINIB DEMONSTRATES COMPLETE INHIBITION OF SYK AND JAK AND RAPID TUMOR RESPONSES IN A PHASE 1 DOSE-RESPONSE STUDY IN PATIENTS WITH RELAPSED/REFRACTORY B CELL MALIGNANCIES
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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 signaling pathways cooperate to control B cell activation. This cooperation appears to be relevant to B cell malignancies as well. IL-4 promotes the survival of BCL cells in culture via up-regulation of MCL1 and BCL-xl, protecting the tumor from death induced by fludarabine and chlorambucil (Steie 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 BCL 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). 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 mononuclear cells (PBMC) and CD19+ B-cell malignancies. A phase II trial in the Netherlands (Phase 2) is now demonstrating efficacy of this triplet combination of a novel anti-CD20 mAb+PI3Kδ+BTK inhibitor (ibrutinib) in pts with B-cell malignancies.

Methods: 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 and relapsed/refractory B-cell malignancies. Expansion cohorts at the highest dose (800mg TGR-1202+full dose ibrutinib) are underway. Future trials for the triplet are warranted.
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 tFL). 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 diarrhea (27%), fatigue (27%) and nausea (24%). Grade 3+ AEs occurring in more than one patient are infection (5 patients), abdominal pain (3 patients) and hypertension (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 with 4 patients in response for greater than 6 months. In addition, 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 these patients had a PR at the end of the 2nd cycle (Figure 1).

**Summary/Conclusions:** Cerdulatinib demonstrates clinical activity in heavily pretreated patients with CLL/B-cell NHL and is generally well tolerated. 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 tFL). 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 diarrhea (27%), fatigue (27%) and nausea (24%). Grade 3+ AEs occurring in more than one patient are infection (5 patients), abdominal pain (3 patients) and hypertension (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 with 4 patients in response for greater than 6 months. In addition, 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 these patients had a PR at the end of the 2nd cycle (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; updates will be presented.
and IRC PET evaluation. Comparison of PFS based on CT-response and re-analysis of PET scans applying the now recommended 5-point scale for PET response assessment will be presented. Pooled analyses of these and data 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 infections were similar in G and R arms for CHOP and CVP but not for B. In all chemo groups, SAEs were more frequent with G than R, and AEs causing treatment discontinuation and fatal AEs were similar. Reductions in T-cell counts were more pronounced and prolonged in the B group than CHOP or CVP groups.

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.
Background: Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma (NHL) 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 δ- and α-isoforms. Aim: We report results from the FL subset of a large phase II study in indolent NHL 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 (60 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 chemotherapy or combination, and 46 (44.2%) with partial response. Stable disease was observed in 35 (33.7%) patients and progression of disease as best response in 2 patients. The median duration of response was 370 days (range 0-687), with 43 responders censored at data cut-off. Median duration of treatment was 22 weeks (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%/24%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/1.4%), hepatic enzymeopathy (AST 28%/1.4%; ALT 23%/1.4%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to copanlisib: one lung infection, one respiratory failure, and one thromboembolic event.

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 370 days. Toxicities were manageable, with a low incidence of severe AEs associated with other PI3K inhibitors, especially hepatic enzymeopathy, 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, aIPI 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 ELOTUXUMAB, LENALIDOMIDE, AND DEXAMETHASONE IN HIGH-RISK SMOLDERING MULTIPLE MYELOMA**

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**Methods:** Patients enrolled on study met eligibility for high-risk SMM based on the newly defined criteria proposed by Rajkumar et al, Blood 2014. Patients who had failed prior therapy were eligible. Patients were then to proceed on maintenance therapy with the combination of elotuzumab, lenalidomide, and dexamethasone for 2 years. Maintenance therapy was then to be continued for 3 years.

**Results:** Of the 64 enrolled patients, 40 continued on study treatment without disease progression for 47.0 months (median duration of follow-up). The overall response rate (ORR) was 86% (61 complete responses (CR), 18 very good partial responses (vgPR), and 15 partial responses (PR)). Median progression-free survival (PFS) was not reached. Median duration of response (DOR) was 25.7 months. Median overall survival (OS) was not reached for patients with NDMM.

**Summary/Conclusions:** The combination of elotuzumab, lenalidomide, and dexamethasone in patients with high-risk smoldering multiple myeloma (SMM) is feasible and active in patients with NDMM, with weekly and twice-weekly ixazomib dosing having been investigated. The study is ongoing.

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**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:** Among other novel agents that have been tested in newly diagnosed 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). In the Phase 2 study (NCT01383928), 204 patients were randomized to receive either ixazomib plus Rd or Rd alone. The median progression-free survival (PFS) for patients who received maintenance therapy was 29.4 months. Median overall survival (OS) was not estimable; 2-year Kaplan-Meier estimate for OS was 92%. A total of 78% of patients who 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 + VGPR + CR) was 94%, the complete response and very good partial response (CR + VGPR) was 86%, and the CR rate was 44%. Two (11%) patients improved their responses during maintenance therapy, and 1 VGPR and 1 VGPR to stringent CR (sCR). Of the 39 patients who received maintenance therapy, 31 had a median follow-up of 13.9 (range 11.0–22.2) months. Median OS was not reached.

**Methods:** Patients with NDMM who received twice-weekly ixazomib (1.6 mg/kg) and lenalidomide (25 mg) received 3 cycles of induction (days 1, 4, 8, 12, and 15) followed by 2 cycles of consolidation (days 1, 4, 8, 12, and 15). Maintenance therapy consisted of twice-weekly oral ixazomib (1.6 mg/kg) and lenalidomide (25 mg) for 2 years. Patients who continued to respond at last were allowed to remain on maintenance therapy with the combination of elotuzumab, lenalidomide, and dexamethasone for 2 years. Maintenance therapy was then to be continued for 3 years.

**Results:** Of the 27 patients who received the combination of elotuzumab, lenalidomide, and dexamethasone (IRd) in the Phase 2 trial, 19 patients (70%) went on to receive maintenance. The median OS was 29.4 months. Median DOR was 25.7 months. Median PFS was not reached. Median OS was not reached for patients with NDMM. The study is ongoing.

**Summary/Conclusions:** The combination of elotuzumab, lenalidomide, and dexamethasone in patients with high-risk smoldering multiple myeloma (SMM) is feasible and active in patients with NDMM, with weekly and twice-weekly ixazomib dosing having been investigated. The study is ongoing. The combination of elotuzumab, lenalidomide, and dexamethasone is active in patients with NDMM, with weekly and twice-weekly ixazomib dosing having been investigated. The study is ongoing.
**Summary/Conclusions:** In patients with NDMM, twice-weekly ixazomib plus Rd resulted in excess adverse 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.

**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≤60mL/min was reported in 67.2% of Za pts and 66.0% of DMB pts. **Figure 1**

**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 for time to first SRE demonstrated a HR(95%CI)=0.81(0.66,0.99), P=0.039 (Figure 1) between DMB and Za. OS was similar between DMB and Za (HR[95%CI]=0.90[0.70,1.16], P=0.41), with fewer deaths with DMB (121[14.1%]) than Za (129[15.0%]). PFS yielded a HR(95%CI)=0.82(0.68,0.99), descriptive P=0.036, with median times of 46.0m (95%CI:34.3,57.8) for DMB and 35.3m (95%CI:30.19,52.9) for Za. The most common TEAes(>20%) for DMB were diarrhea and nausea. The rates of SAEs (DMB,ZA [%];46.0,47.3), hypocalcemia (16.9,12.4; serious:0.9,0.2), and positively adjudicated ONJ (4.1,2.8) were comparable to known safety profiles. Fewer DMB pts (%) compared with Za pts had AEs potentially related to renal toxicity (10.0,17.1;P<0.001), most notably in pts with baseline CrCl<60mL/min (12.9,20.4). TEAes led to IP discontinuation in 12.2% of pts (12.9,11.5).
Background: Pembrolizumab (pembro) is a humanized, highly selective, high-affinity IgG4/κ antibody that blocks the interaction between programmed death-1 (PD-1) and its ligands PD-L1 and PD-L2, activating tumor immunity. Pembrolizumab plus lenalidomide (len) 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.

Aims: To determine the maximum tolerated dose (MTD) and safety and tolerability of pembrolizumab plus len and low-dose dex in patients with RRMM. Additionally PD-L1 and PD-L2 expression in bone marrow (BM), immune profiles in circulating lymphocytes, and gene expression in blood were evaluated.

Methods: This open-label, phase 1 KEYNOTE-023 (NCT02036502) study of pembrolizumab plus len and low-dose dex enrolled 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), len 25 mg orally on days 1-21, and dex 40 mg orally weekly on each 28-day cycle. Primary end points were safety and determination of the MTD. Ongoing follow-up was focused on maintaining hematocrit (HCT) level ≥45%. Response assessment was performed at weeks 8 and 28. Immuno-oncology Biomarker Analysis (IBA) was performed at screening, or before the first dose of study drug. Absolute and/or relative numbers of circulating lymphocytes (by flow cytometry [FC]) and gene expression profile (GEP) (by Neoan) were evaluated in predose; cycle 1, day 1 (C1D1); and cycle 2, day 1 (C2D1) blood samples.

Results: MTD was determined as pembrolizumab 200 mg IV Q2W plus len 25 mg and dex 40 mg. Median (range) age was 61 years (46-77); median (range) number of prior lines of therapy was 4 (1-10); 38 (75%) patients were len-refractory, and 27 (53%) were double-refractory. Most common grade ≥3 treatment-related adverse events (TRAEs) were neutropenia (33%), thrombocytopenia (18%), and anemia (12%). Two patients (4%) died because of TRAEs (hepatic failure, ischemic stroke). Immune-related adverse events occurred in 5 (10%) patients. No pneumonitis was reported. ORR was assessed by IMWG 2006. Exploratory biomarker analyses included analysis of PD-L1 and PD-L2 on CD3+CD138+ cells in BM aspirate samples obtained at screening, or before the first dose of study drug. Absolute and/or relative numbers of circulating lymphocytes (by flow cytometry [FC]) and gene expression profile (GEP) (by Neoan) were evaluated in predose; cycle 1, day 1 (C1D1); and cycle 2, day 1 (C2D1) blood samples.

Old and new drugs in MPN

RUXOLITINIB FOR THE TREATMENT OF INADEQUATELY CONTROLLED POLYCYTHEMIA VERA WITHOUT SPLITENOMEGALY: 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 indicated by increasing hematocrit (HCT) levels (HCT ≥45%). Ruxolitinib (RUX) is a JAK1/2 inhibitor that reduces HCT, erythrocytosis, and symptom burden. The RESPONSE-2 trial evaluated the efficacy and safety of RUX vs best available therapy (BAT) in hydroxyurea (HU)-resistant/intolerant pts with PV ≥18 years without splenomegaly and with phlebotomy (PT) requirement to control HCT. At week (wk) 28 (primary analysis), HCT control was reported in 46/74 pts in the RUX arm vs 14/75 pts in the BAT arm. The aim of this preplanned analysis of RESPONSE-2 was to evaluate the duration of efficacy and safety of RUX vs BAT, after all pts reached wk 80 into the study or discontinued the study.

Methods: Pts were randomized 1:1 to RUX 10 mg twice daily or BAT. Primary end point was the proportion of pts who achieved HCT control at wk 28 (absence of PT eligibility [HCT >45], ie, ≥3 percentage points from baseline, or HCT <48%) from wk 8 to 28, with ≥1 pt eligible for HCT at wk 0 to 8. Key secondary end point was the proportion of pts who achieved complete hematologic remission at wk 28 (CHR: HCT <45%, WBC ≤10 ×10^9/L, platelet count ≤450 ×10^9/L). In a post hoc analysis, pts ≥65 years of age were included. The analysis was performed on all pts with ≥1 pt-week of exposure (primary analysis) or ≥4 pt-weeks of exposure (post hoc). The post hoc analysis was performed per-protocol (n=75) and as intention to treat (n=109) analyses.

Results: Baseline demographics were comparable among RUX (N=74) and BAT (N=75) arms. At baseline (wk 0) 28/32 pts (87%) in the BAT arm and 25/32 pts (78%) in the RUX arm were still receiving Tx; while 5 pts discontinued Tx (adverse events [AEs]=3 pts, physician’s decision/patient withdrew consent=1 pt, each). In BAT arm, 58 pts crossed over to RUX (crossover data to be included in presentation) with remaining pts either ongoing follow-up (fu; n=59) or having discontinued Tx (completed fu per protocol, n=7; death, n=1; other reasons, n=4). Median exposure was 28.4 wk in the BAT arm and 28 wk in the RUX arm. At wk 80, durable HCT control was achieved in 35 pts (47%) in RUX vs 2 pts (3%) in BAT arm. Of those who achieved a HCT response at wk 28, Kaplan-Meier estimate of maintaining response up to wk 80 was 78.3% in the RUX arm. Durable CHR was achieved in 18 pts (24%) in RUX vs 2 pts (3%) in the BAT arm. Total number of pts was higher in the RUX arm vs BAT arm (Figure 1). At wk 80, 45% of pts randomized to RUX continued to achieve ≥25% of reduction in the MPN-SAF TSS. At wk 80, mean percentage change from baseline in JAK2V617F allele burden was −9.7% in the RUX (n=65) vs +0.3% in the BAT arm (n=3). AEs observed were consistent with those generally reported with RUX (primarily grade ≥3). Most common AEs (all Grades) were headache (16.9%), arthralgia (9.1), and pruritus (9.1) in the RUX arm vs pruritus (37.5%), headache (16.9), and thrombocytopenia (15.0) in the BAT arm. Rate of thromboembolic events (Standardized MedDRA Query, exposure-adjusted) was RUX (1.5) vs BAT arm (1.9). No pts in the RUX arm had disease progression or ≥2 pts in the BAT arm. No deaths were reported in the RUX arm vs 3 pts in the BAT arm (septic shock/disease progression/study indication=1 pt, each).

Summary/Conclusions: RUX provided durable HCT control, durable CHR, reduction in PT requirement, improved symptom burden, and was generally well tolerated with <90% of pts still receiving Tx at wk 80. This modest reduction in allele burden over time. Findings from both RESPONSE studies suggest RUX could be considered as a standard of care for second-line Tx in this inadequately controlled pt population with PV.
PHASE 3 RANDOMIZED TRIAL OF MOMELOTINIB VERSUS RUXOLITINIB IN JAK INHIBITOR NAIVE PATIENTS WITH MYELOFIBROSIS: RESULTS OF THE SIMPLIFY-1 STUDY
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Background: Momelotinib (MMB), an investigational oral JAK inhibitor (JAKI), 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 response, 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; palpable spleen ≥5cm; and no Grade ≥2 peripheral neuropathy. Informed consent was obtained. Stratification was by transfusion dependence and platelets (<100K, 100K-200K, and >200K/μl). Patients were randomized 1:1 to 24 weeks of 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 all 144 patients included ruxolitinib, and 27% of patients were on ruxolitinib in combination with other drugs. Efficacy results are in Table 1. The most common treatment-emergent adverse events in MMB patients were diarrhea (33%), asthenia (19%), nausea (19%), and cough (17%), and in BAT patients, asthenia (21%), fatigue (19%), anemia (15%), diarrhea (15%), and abdominal pain (15%); the most common Grade ≥3 adverse events in MMB 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>
<th>BAT</th>
<th>p-value</th>
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<tbody>
<tr>
<td>SRR, %</td>
<td>67</td>
<td>57</td>
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<tr>
<td>TSS RR, %</td>
<td>26.2</td>
<td>5.9</td>
<td>&lt;0.001*</td>
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<td>Transfusion rate (units/month)</td>
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<td>1.3</td>
<td>0.39</td>
</tr>
<tr>
<td>TI rate, %</td>
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<td>21.2</td>
<td>0.001*</td>
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<tr>
<td>TD rate, %</td>
<td>50.0</td>
<td>63.5</td>
<td>0.10</td>
</tr>
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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.

S787

MOLECULAR RESPONSE TO HYDROXYUREA AND ROPEGINTERFERON ALFA-2B IN THE PROUD-PV RANDOMIZED PHASE 3 TRIAL

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Background: Interferon alfa (IFNa) has been successfully used to treat myeloproliferative neoplasms (MPN) for many years and several phase 2 studies have indicated recently 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 report the findings of a randomized controlled phase III trial (PROUD-PV) comparing the novel, long-acting Rogepinginterferon 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: Randomized, controlled, multicenter phase 3 trial comparing efficacy, safety and tolerability of hydroxyurea and Rogepinterferon 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 hematological response (CHR) according to ELN criteria and normal spleen size. As an important secondary endpoint the treatment of %JAK2V617F was assessed as rate of complete and partial molecular response (CPMR) according to modified ELN criteria. In the group of pts enrolled in France, we could study BM progenitors clonogenic potential by cultures with or without Erythropoietin (EPO) at baseline and after 12 months of therapy. The presence of colonies without EPO, namely Endogenous Erythroid Colonies (EECs) is a hallmark of PV. After 14 days, erythroid colonies were enumerated and picked for molecular analyses.

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 and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46.2% at baseline, 24.0 (95% CI, 16.7-31.3) and 35.6 (95% CI, 28.1-43.0) at 12 mos. Complete remission was achieved at 12 mos, but PPR was observed in 40% and 25% of pts in AOP2014 and HU arms (p>ns), respectively. BM 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 (median decrease of 64%) between samples collected at baseline and after 12 months of therapy compared to HU (median decrease 25%). In addition, clonal architecture studies showed that the % of JAK2V617F mutant colonies before and after treatment profoundly decreased in all AOP2014-treated patients while wild type JAK2K colonies decreased from 86% at baseline to 46% 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 didn’t significantly decrease (from 87% at baseline to 79% after 12 mos).

Summary/Conclusions: In this phase 3 trial comparing Rogepinginterferon alfa-2b versus HU, we found a different impact of both drugs on hematopoietic cells. Although both treatment induced a decrease of JAK2K mutant allele burden at 12 mos in peripheral blood, BM clonogenic assays suggest that AOP2014 is able to specifically target JAK2K 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.

S788

POOLED SURVIVAL ANALYSIS OF MIDOSTAURIN CLINICAL STUDY DATA (D2201+A2213) IN PATIENTS WITH ADVANCED SYSTEMIC MASTOCYTOSIS COMPARED WITH HISTORICAL CONTROLS


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Background: AdvSM (ie, aggressive SM [ASM], SM with an associated hematologic neoplasms [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 advSM. 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 large, international, multicenter study. 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 selection, subgroups analyses, and mulivariate analyses were performed to assess whether baseline 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 in patient selection was conducted using the start date of last treatment to death.

Results: The primary analysis of OS in patients with advSM 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=0.0204; 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=0.381 [95% CI, 0.169-0.960]; P=0.101). Subgroup analyses of OS showed HR in favor of midostaurin for all subgroups analyzed (HR from the multivariate analysis=0.38 [95% CI, 0.22-0.65]; P=0.0004).

Table 1.
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≥10−4 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%) albeit not 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), OS (80±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=58), no significant differences were observed regarding EFS (81±5% vs 71±5%, p=0.27), OS (98±3% vs 93±3%, p=0.16) and CI of ML-DS (19±6% 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 remains to be a threat 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.

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 around a 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≥10−4 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%) albeit not 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), OS (80±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=58), no significant differences were observed regarding EFS (81±5% vs 71±5%, p=0.27), OS (98±3% vs 93±3%, p=0.16) and CI of ML-DS (19±6% 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 remains to be a threat 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.
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 received bolus days 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); or allo-SCT if leukocyte index [LI=leucocytes x (BM blasts/100)] ≥20 or high dose cytarabine (HDAC) (one course) if LI <20. Intermediate risk (IR), defined as patients in CR after a single induction course, <50x10E9/l white blood cells at diagnosis, normal karyotype and absence of FLT3-ITD internal tandem duplication (FT3-ITDwt) and no MLL rearrangement: ASCT. Adverse risk (AR), patients not included in FR or IR: CR or ASCT or allo-SCT as first line treatment (allo-SCT) depending on donor availability (HLA-identical sibling or unrelated donor if high risk of relapse).

Results: There were 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. FT3-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 was comparable between normal (CR = 91% in NPM1 mutation without FLT3-ITD, 77% in intermediate cytogenetic and no mutations, 74% if FT3-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. Separately serial immunophenotypic quantitation of leukaemic stem/progenitor cells (LSC) was performed in 44 patients.

The RAVVA trial randomized 259 adults with AML (n=217) and MDS (n=42) to receive AZA monotherapy (AZA (75 mg/m²) x 7 days every 28 days) or AZA combined with VOR (300 mg orally per day) for a minimum of 6 cycles. Next generation sequencing was performed on 42 genes commonly mutated in AML and MDS in 250 patients treated on the RAVVA trial and correlated with response. Separately serial immunophenotypic quantitation of leukemic stem/progenitor cells (LSC) was performed in 44 patients.

S972
SORAFENIB MAINTENANCE IN FLT3-ITD MUTATED ACUTE MYELOID LEUKAEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANT
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Background: The fms-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) mutation is a genetic alteration found in approximately 30% of patients with acute myeloid leukemia (AML). Although autologous transplantation with AZA achieves remission rates similar to those with FLT3-ITD 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 patients requiring non-progression. Sorafenib(SFB) is a TKI with activity against RAF, VEGF and FLT3-ITD and its use as maintenance therapy after allogeneic SCT has been shown as a promising approach to decrease relapse. Several studies report that SFB maintenance post SCT provides durable complete responses; however, there are also descriptions of sorafenib post SCT triggering acute GVHD, cytokopenia, rash and thrombocytopenia.

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 leukemia (acquired SCT between 2010-2016), who achieved remission after induction therapy with AZA, followed by a minimal disease state and transplantation. All patients had FLT3-ITD mutation that underwent ASCT 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

S971
MOLECULAR PREDICTORS OF RESPONSE TO AZACITIDINE THERAPY: THE RESULTS OF THE UK TRIALS ACCELERATION PROGRAMME RAVVA STUDY
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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 (CRn) in 69% while it was active disease in 31%. PFS at 24 months post SCT was 82% in the maintenance and 45% in control groups HS: 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 cytopenias, liver function test abnormalities, and fatigue.

Figure 1.

Summary/Conclusions: Sorafenib maintenance is safe and can produce long term durable remissions after allogeneic 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) remissions, 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 4.1 wks for ANC (≥1K) and 5.9 wks for platelets (≥100K) in patients who achieved CR/CRI. Patients had intermediate (48%) or adverse (36%) cytogenetic risk. 16% had secondary AML. The median time to count recovery from D1 of therapy was 4.1 wks for ANC (≥1K) and 5.9 wks for platelets (≥100K) in patients who achieved CR/CRI. Four patients had hematologic DLTs, 1 at 30 and 3 at 40 mcg/kg. Non-hematologic TEAEs were consistent with those seen in the D1 and 4 schedule. Of the 24 EE patients, best responses included 12 CR (50%), 6 CRi (25%), and 3 mLFS (13%) with a CRc rate of 75%, achieved in 1st cycle. Of the evaluable patients with blast clearance, 89% (17/19) achieved MRD negative status. Across schedules (N=67), the CRc rate was 76%; 79% (44/56) of evaluable patients with blast clearance achieved MRD negativity. The CRc rate at the 40 mcg/kg dose level was 91% (10/11); all 11 patients had blast clearance and 90% (9/10) of evaluable patients achieved MRD negative status. The median time to count recovery from D1 of therapy was 4.1 wks for ANC (≥1K) and 5.9 wks for platelets (≥100K) in patients who achieved CR/CRI. Four patients had hematologic DLTs, 1 at 30 and 3 at 40 mcg/kg. Non-hematologic TEAEs were consistent with those seen in the D1 and 4 schedule. Of the 24 EE patients, best responses included 12 CR (50%), 6 CRi (25%), and 3 mLFS (13%) with a CRc rate of 75%, achieved in 1st cycle. Of the evaluable patients with blast clearance, 89% (17/19) achieved MRD negative status. Across schedules (N=67), the CRc rate was 76%; 79% (44/56) of evaluable patients with blast clearance achieved MRD negativity. The CRc rate at the 40 mcg/kg dose level was 91% (10/11); all 11 patients had blast clearance and 90% (9/10) of evaluable patients achieved MRD negative status. The CRc rate at the 40 mcg/kg dose level was 91% (10/11); all 11 patients had blast clearance and 90% (9/10) of evaluable patients achieved MRD negative status. The CRc rate at the 40 mcg/kg dose level was 91% (10/11); all 11 patients had blast clearance and 90% (9/10) of evaluable patients achieved MRD negative status.

Summary/Conclusions: S33A 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.
**21-COLOR FLOW CYTOMETRY REVEALS IMMUNOPHENOTYPES ASSOCIATED WITH RESPONSE IN ACUTE GRAFT-VERSUS-HOST DISEASE PATIENTS TREATED WITH THE JANUS KINASE INHIBITOR INCB039110**

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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, INCB039110) 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 INCB039110 for aGVHD (NCT02614612). Preliminary results were previously presented at ASH 2016 (Schroeder et al).

**Methods:** Patients (n=30) were >18 years old undergoing first alloHSCT from any source with steroid-refractory or treatment-naive grades IIb-IVD aGVHD, randomized 1:1 to 200 or 300 mg oral daily INCB039110 combined with corticosteroids. Peripheral blood, obtained at treatment days 7, 14, 28, 56, 100, and 180, was analyzed by 21-color FACS quantifying >30 cell types, including B, CD4+ and CD8+ T, myeloid, monocyte-derived suppressor cells (MDSCs), natural killer cells (NKs), and monocytic and plasmacytoid dendritic cells (DCs). Patients were stratified by treatment response (e.g. complete response (CR), partial response (PR), mixed response (MR)).

**Results:** During INCB039110 treatment, overall B, T, and myeloid proportions did not correlate with response. However, the CR group increased native KCs (CD3-CD20-CD4-HLA-DR-CDS6+), mDCs (CD3-CD20-CD14-HLA-DR+CD11c+), and memory CD4+ T cells (CD3+CD4+CD45RA-). Among CD4+ memory cells, the CR group showed significant or trend-toward-significant increases in Th1 (CXC5-CR6-CR10-CR3+), Th2 (CXR5-CR5-CR6+CCR4+CCR3-CR10-), Th17 (CXC5-CR5-CR6-CR10-CR3+), and Th22 (CXC5-CR5-CR6+CCR4+CCR3-CR10+). Tregs (CD4+CD25+CD127+) trended toward a ~2-fold increase in the CR group. Within the monocyte subgroup (CD3-CD20-CD14+), the CR group skewed toward classical monocytes (HLADR+CD16+) (64.7% vs 36.0%, CR vs PR/MR, p=0.0078) and away from MDSCs (HLADR-CD16+) (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, finding consistent for both CD16+ and CD16- NK cells (Fig. 1 a, b). Before treatment, decreased native 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-CR6-CR10+) 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 native T cells may predict better outcomes in INCB039110-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.

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**INCB039110 DISEASE PATIENTS TREATED WITH THE JANUS KINASE INHIBITOR ASSOCIATED WITH RESPONSE IN 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 by MRSA and MRGNB in development of grade III-IV, patients were colonized with more than one MDR pathogen. The cumulative incidence (CI) of severe (grade III-IV) acute GVHD was significantly higher in patients colonized with MDR-GNB (27%, 95% CI, 19-39%) than in non-colonized patients (14%, 95% CI, 7-23%) (p=0.04). Moreover, MDR-GNB colonized patients had significantly more gas troenteritis (GI) GVHD CI as opposed to non-colonized patients (28% (95% CI, 20-41%) vs 14% (95% CI, 7-23%), p=0.02) and more acute GVHD-related mortality (16% (95%CI, 9-26%) vs 7% (95%CI, 3-15%), p<0.10). A substantial and independent role of gut colonization with MDR-GNB on the development of acute GVHD was confirmed by multivariate analysis using time-dependent covariate functions for high risk disease, myeloablative conditioning, peripheral blood stem cells, unrelated donor (hazard ratio 2.14; 95%CI, 0.99-4.68, P=0.05), older age (hazard ratio 2.15; 95%CI, 1.00-4.59, P=0.04) and 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 GI GVHD. With growing resistance and lack of efficient antibiotics, decolonization strategies as fecal microbiota transplantation become an attractive strategy for restoration of healthy gut flora and prevention of severe acute GVHD.

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**IMPACT OF HLA DISPARITY ON OUTCOME IN HLA-HAPLOIDENTICAL BONE MARROW TRANSPLANTATION FOLLOWED BY HIGH DOSE POST-TRANSPLANT CYCLOPHOSPHAMIDE**

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**Background:** By definition ‘haplo-identical’ donors share genotypically 4/8 anti-
gens with recipients. However, causal phenotypical homozygosity in the non-
shared haplotypes made the real degree of disparity less than 4/8 in a few donor/recipient pairs.

Aims: Since 2010, patients who lacked a HLA-identical donor have been trans-
planted from a haploidentical donor in our two Italian institutions. In this large series of patients we aim to verify the real degree of antigen disparity between donor/recipient pairs and whether it impacts on transplantation outcomes.

Methods: All haploplants performed in two Italian institutions from August 2010 to July 2016 (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 cyclosporine 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 reso-
lution level, as defined by EFL standards and by NGS at allelic level in 2016 for the same loci. When applicable (72.3% of patients) members of the immediate family where typed to definitively establish HLA genotype and haplotype identity. Differences in HLA mismatching were calculated using HVG direction and graft rejection rate according to the degree of HLA mismatches in the HVG direction.

Results: Median age of patients was 48 years (17-74). Diagnoses included acute myeloid leukemia (130), acute lymphoblastic leukemia (64), lymphoid and myeloid leukaemia (43), 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%. Considering the proportion of “true” hap-
loidentical D/R pairs, 231 out of 318 (72%) couples showed 4/8 mismatches at HL A and HLA DRB1 loci. Neither OS nor NRM showed significant correlation with the degree of overall mismatches at 2 years (0-2 mismatches: 54.2% vs 3-4 mismatches: 58.8%, p = 0.08 and 0-2 vs 3-4: 19.1%, p = 0.93, respectively).

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.

S797
CYCLOPHOSPHAMIDE VERSUS ETOPOSIDE IN COMBINATION WITH TOTAL BODY IRRADIATION AS CONDITIONING FOR ADULTS WITH ACUTE LEUKEMIA WORKING PARTY OF THE EBMT
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Background: High-risk acute myeloid leukemia (AML) is mainly defined by the presence of determined poor-risk cytogenetic abnormalities and is a stan-
dard indication for allogeneic stem cell transplantation (SCT). Nevertheless, high-risk AML is a very heterogeneous group including several abnormalities with different levels of prognostic impact. Deletion 5q or monosomy 5 (-5q-) has been part of the high-risk group of AML for many years. SCT seems to improve their survival but the autologous impacts of other high-risk cytogenetic features on survival have never been thoroughly studied.

Aims: To evaluate the role of SCT in -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 (UD) were included in the analysis. Peripheral blood was used as a source of stem cells in 62% of the patients. Conditioning regimen was myeloablative in all cases (the median TBI dose was 12Gy); 1346 patients were treated with CYTBI while 152 patients with Vep/TBI. Patients in the Vep/TBI group were younger (median 28 y. vs 30 y., p = 0.04), treated more in recent period (median year of HCT: 2009 vs 2007, p = 0.009) and treated more frequently in CR1 (87% vs 78%, p = 0.01).

Results: In a univariate analysis, as compared to CYTBI, the use of Vep/TBI was associated with significantly reduced incidence of relapse (17% vs 30% at 5 years, p = 0.007), increased rate of leukemia-free survival (LFS, 60% vs 50%, p = 0.04) as well as improved “GVHD and relapse-free survival” (GRFS, 43% vs 33%, p = 0.04). No significant effect could be observed in terms of the incidence of non-relapse mortality, acute or chronic GVHD. In a multivariate model the use of Vep/TBI was associated with reduced risk of relapse (HR=0.62, p=0.04) while the effect on other study end-points was no longer significant. Among other factors, recipient age (HR=1.17 per every 10 years, p<0.0001), year of alloHCT (HR=0.97 per every year), p=0.01) and disease stage (HR=2.14 for CR2, p<0.0001) had significant influence on the risk of treatment failure, either relapse or non-relapse mortality. The risk of relapse was additionally increased for sibing vs unrelated donor transplants (HR=1.47, p=0.01) and donorrecipient gender combination other than female/male (HR=1.26, p=0.04).

Summary/Conclusions: Conditioning regimen based on etoposide combined with TBI appears more effective than the cyclophosphamide TBI combination for adult patients with Ph-negative ALL treated with alloHCT. Further, prospective studies are needed to confirm our observation and potentially discriminate subgroup of patients who are most likely to benefit from the use of etoposide.
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, CK, or abn(17p) (N=90). Group 3 (MK) included 169 pts with -5/5q- and CK but no MK or abn(17p) (N=90). Group 3 (MK) included 169 pts with -5/5q- and CK but no MK or abn(17p). Finally, group 4 (17p) included pts with -5/5q- and abn(17p) (N=193). The 4 groups were quite similar in term of characteristics. 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). OS decreased also significantly from group 1 to 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 CK, MK 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.
Background: the outcome of Ph+ acute lymphoblastic leukemia (Ph+ ALL) has drastically improved since the introduction of tyrosine kinase inhibitors (TKI). At present however, well-defined prognostic markers, beyond the monitoring of minimal residual disease (MRD) during follow-up and to a lesser extent IKZF1 deletions, are lacking.

Aims: To identify genomic lesions of prognostic value, we evaluated copy number aberrations (CNA) by SNP arrays, confirmed them by multiplex ligation-dependent probe amplification (MLPA) and we set up a droplet digital PCR (ddPCR) assays for additional lesions. Furthermore, we correlated the lesions identified with MRD monitoring, outcome and biological features, including reports of the differential use of type of fusion protein (p190 or p210). Finally, in a subset of patients gene expression profiling (GEP) was carried out.

Methods: Genomic DNA of 116 newly diagnosed adult Ph+ ALL patients enrolled in 4 consecutive GIEMMA trials, namely 021B1, 09B4, 1205 and 1509, was evaluated. All the trials were based on an induction with steroids and TKI, the first 2 with imatinib and the remaining with dasatinib. For CNA, the Cytoscan HD Arrays (Affymetrix, Santa Clara, CA) were used. The lesions were confirmed by MLPA on all samples using the Mala SNP MLPA P335-A3 ALL-IKZF1 kit (MRC-Holland, Amsterdam, The Netherlands), ddPCR was used to validate lesions targeting MEF2C. In 42 cases, GEP experiments were performed using the HGU133 Plus 2.0 gene chips (Affymetrix, Santa Clara, CA).

Results: We found a similar load and type of lesions across the 4 trials, one of which included elderly. The majority of lesions targeted IKZF1 (84%), PAX5 (36%) and CDKN2A/B (32%). In our cohort, IKZF1 deletions alone did not affect complete molecular response (CMR) achievement or relapse-free survival, whereas patients harboring CDKN2A/B and PAX5 deletions had a significant inferior outcome (p=0.004, p=0.003 respectively). In line with this, a worse DFS was observed for the so-called “IKZF1 plus” cases, i.e. concomitant deletions of IKZF1 and CDKN2A/B and/or PAX5 (46% vs 24% at 36 months, p=0.005). MLPA confirmed the incidence of these deletions and allowed the study of IKZF1 isoforms. Among IKZF1 deleted cases, patients carrying the Δ4-7 isoform (25%) had a worse DFS (p=0.02) than patients harboring other IKZF1 isoforms. Importantly, SNP arrays highlighted novel genomic lesions targeting MEF2C in 13% of cases, which were associated to the achievement of a CMR (p=0.05) and had a significant impact on DFS (62% vs 32% at 36 months, p=0.02). The association with CMR was not affected by the trial (p=0.76) or the TKI used (p=0.57). This result was confirmed by ddPCR. Unsupervised hierarchical clustering of GEP experiments identified 3 subgroups: the first comprised mainly patients who reached a CMR, the second one the patients who had IKZF1 alone, and the last one comprised “IKZF1+MEF2C” patients. GEP analysis showed an overexpression of genes involved in cell communication and protein modification process in PAX5 deleted cases, suggesting that these genes could be contributing factors in BCR/ABL1-driven leukemogenesis.

Summary/Conclusions: In adult Ph+ ALL, IKZF1 deletions have a prognostic impact and could be considered among other lesions. Among IKZF1 deletions, only the Δ4-7 deletion has a deleterious effect. MEF2C lesions carry prognostic implications, being significantly associated with a better prognosis. This study paves the way to design a prognostic model for adult Ph+ ALL that includes these findings and more conventional features, in order to better stratify patients at diagnosis and to further optimize treatment.
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(9;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.

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.

Table 1.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>n=115</th>
<th>Disease characteristics</th>
<th>n=115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at registration median (range)</td>
<td>49 (30-65)</td>
<td>B-ALL</td>
<td>100 (1-3)</td>
</tr>
<tr>
<td>Preexisting WBC in median (range)</td>
<td>8.6 (0.057-77.8)</td>
<td>T-ALL</td>
<td>1.5 (1.3)</td>
</tr>
<tr>
<td>Sex (%)</td>
<td>61 (53)</td>
<td>M</td>
<td>39 (33)</td>
</tr>
<tr>
<td>Male</td>
<td>54 (47)</td>
<td>MRD</td>
<td>6 (5.2)</td>
</tr>
<tr>
<td>Female</td>
<td>40 (34)</td>
<td>Complex karyotype</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Sibling</td>
<td>40 (34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor-type</td>
<td>61 (53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matched-identical</td>
<td>33 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matched-related</td>
<td>75 (65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Induction MRD N (%)</td>
<td>62 (53.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>38 (33)</td>
<td>Unknown</td>
<td>50 (43.4)</td>
</tr>
<tr>
<td>Negative/POQR</td>
<td>77 (67)</td>
<td>High-Risk</td>
<td>35 (15.6)</td>
</tr>
<tr>
<td>Negative/POQR</td>
<td>77 (67)</td>
<td>High-Risk</td>
<td>35 (15.6)</td>
</tr>
<tr>
<td>Positive</td>
<td>38 (33)</td>
<td>Unknown</td>
<td>50 (43.4)</td>
</tr>
</tbody>
</table>

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 Euro-Clonality-NGS Consortium.

Research Support: Amgen.

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 days with 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 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.

Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median (IQR range)</th>
<th>Median (IQR range)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of neutropenia</td>
<td>14 (9.5-24)</td>
<td>11 (8-21)</td>
<td>0.006</td>
</tr>
<tr>
<td>Days of fever*</td>
<td>4 (2-8)</td>
<td>4 (2-8)</td>
<td>0.69</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>18 (12.5-215)</td>
<td>10 (9.7-20.2)</td>
<td>0.0047</td>
</tr>
<tr>
<td>Per protocol population</td>
<td>EG (n=63)</td>
<td>CG (n=68)</td>
<td>0.25</td>
</tr>
<tr>
<td>Days of fever</td>
<td>4 (1-14)</td>
<td>5 (2-8)</td>
<td>0.16</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>19 (14-22)</td>
<td>14.5 (7.8-20)</td>
<td>0.16</td>
</tr>
<tr>
<td>Days of fever*</td>
<td>3 (1-7.2)</td>
<td>3.5 (1.5-7)</td>
<td>0.68</td>
</tr>
<tr>
<td>ITT: Intention to treat; EAT: empirical antimicrobial therapy; EG: experimental group; CG: control group; IQR: interquartile range.</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 1.
Summary/Conclusions: In hematological patients with febrile neutropenia of unknown origin the discontinuation of empirical antibacterial therapy after 72 hours of apyrexia 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 after vaccination. Analyses for each of the 12 pneumococcal serotypes common for PCV13 and PPSV23 were performed by opsonophagocytosis 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 8 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 significantly higher for PCV13 than for 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 as possible 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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1IRCCS San Raffaele Scientific Institute, 2Vita-Salute San Raffaele University, Milan, Italy

Background: Infection-related mortality (IRM) is a major challenge after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Aims: 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). A total of 273 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-haploidentical, 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 T or B-cell depletion or from previous colonization 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 2-28) 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). In the prospective validation set, only 100-day IRM was calculated due to a shorter follow-up, being of 0%, 3% (95% CI 0-13) and of 14% (95% CI 3-33) for low, intermediate and high-risk patients (P=0.003). Additionally, in both training and retrospective validation sets (n=492), the 2-yrs OS was different among the 3 groups being 59% (95%CI 52-67), 50% (95% CI 43-59) and 37% (95% CI 29-48) for low, intermediate and high-risk groups, respectively (P=0.0001). In the prospective validation set, only 100-day OS was evaluated, being of 95% (95% CI 88-100), 91% (95% CI 82-100) and 80% (95% CI 65-100), respectively (P<0.001). Out of a total of 129 infection-related deaths, 94/129 (73%) were attributed to bacteria, 22/129 (17%) to viruses, 11/129 (8%) to fungi and 2/129 (2%) to parasites.

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 in currently on the way for the external validation of these results.

S807

LETTERMOVIR FOR PREVENTION OF CYTOMEGALOVIRUS INFECTION IN ADULT CMV-SEROPPOSITE RECIPIENTS OF ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: CMV remains a common complication of HCT, yet no antiviral drug suitable for prophylaxis is available in HCT. Letermovir is a first-in-class drug
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, 9% through Week 14 (Day +100) post-HCT, 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% haploidential 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.

### S808

**EFFICACY AND SAFETY OF DEFIBROTIDE TO TREAT HEPATIC VENO-OCCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME POST-CHEMOTHERAPY: A POST HOC ANALYSIS OF FINAL DATA 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), 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-HSCT 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 hematochezia (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 hematochezia, nausea, encephalopathy, epistaxis, or hypotension (2% each). Related 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.
Iron: Deficiency and overload

S809

LACK OF THE FERROPTOSIS INHIBITOR GPX4 IN ERYTHROID CELLS CAUSES A BLOCK IN RETICULOCYTE MATURATION AND A HYPOXIC SIGNATURE WITH IMPAIRED HEPCIDIN REGULATION

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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 days 3.5 by the occurrence of tissue-specific ablation in neurons and T-cells causing neurodegeneration 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 hepcidin 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 a colorimetric assay. All animal experiments were approved by the institutional guidelines 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 and EPO measurement revealed a strong increase in this population, suggesting that the erythropoiesis could be due to a block in the reticulocyte maturation. Reticulocyte FACS characterization revealed a shift towards a more immature population while tissue electron microscopy analysis showed an accumulation of unphagocytosed vesicles containing remnants of mitochondria. Analysis of the spleen revealed extramedullary erythropoiesis. The anaemia and the erythropoiesis trigger a hypoxic signature hallmarkned 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 production. 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 down-regulation upon ErFe stimulation. This finding opens new insights in the mechanism that regulate hepcidin during hypoxia.

S810

IDENTIFICATION OF GUANOSINE 5′-DIPHOSPHATE AS POTENTIAL IRON MOBILIZER: PREVENTING THE HEPCIDIN-FERROPORTIN INTERACTION AND MODULATING INFLAMMATORY RESPONSES INSTIGATED BY INTERLEUKIN-6/STAT-3 PATHWAY

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Background: Anemia of inflammation (AI) is one of the most common manifestations of iron deficiency in the patients with inflammatory conditions. AI is responsible for hypoferremia, with consequent iron-restricted erythropoiesis with high level of hepcidin, which stimulate the internalization of ferroportin (FPN) transporter. Therefore, inhibiting hepcidin-mediated FPN degradation can be an important strategy to ameliorate AI.

Aims: To increase iron bioavailability we selected a Novel compound against hepcidin action through natural compound libraries that might provide a new alternative approach to increase iron absorption for prevention of hepcidin-mediated FPN internalization and to ameliorate turbine-induced anemic state with different insilico, invitro and invivo studies.

Methods: To find a systemic approach involving in silico, in vitro and in vivo studies was employed to identify hepcidin inhibiting agents. To identify a potent hepcidin-binding agent, natural compounds were screened using molecular docking and dynamics simulations and further investigated on cell lines (GFP-FPN, Caco-2, HepG2) using flow cytometry and western blotting. Normal or turpenline induced anemic mice were used in the associated studies.

Results: The virtual screening via molecular modelling showed that GDP as a potent hepcidin-binding agent as shown in the (Figure 1A). In vitro studies revealed that GDP significantly increased ferroportin stabilization in GFP-FPN cell lines (Figure 1C) and in vivo results showed that co-administration of GDP and ferrous sulphate (FeSO4) significantly improved the turbine-induced anemic state with increase in haemoglobin level (Figure 1B).

Figure 1.

Summary/Conclusions: AI is a normocytic anemia, common among patients with chronic infection and inflammatory disorders. We found that GDP molecule have higher contribution to the stability of hepcidin-GDP complex and thus blocks its interaction with FPN. The results support the novel hypothesis that GDP along with iron supplement regimen can overcome the binding of hepcidin from interaction with FPN that would be an effective treatment for AI.

S811

UNRAVELING THE MOLECULAR PATHOGENESIS OF INEFFECTIVE ERYTHROPOIESIS IN CONGENITAL DYSERYTHROPOIESIS ANEMIA TYPE II: A LITERATURE REVIEW AND PROSPECTIVE EVALUATION OF RAP-011 TREATMENT

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Background: Congenital Dyserythropoietic Anemias (CDAs) are subtypes of bone marrow failure syndromes, hallmarkled by ineffective erythropoiesis. The most common form is CDA type II (CDAII), showing moderate/severe anemia, relative reticulocytopenia, jaundice, and iron overload. It is inherited as autosomal recessive disorder to loss of function mutations in SEC23B gene. More than 300 CDAII cases and 80 causative mutationshave been described so far. Despite this high allelic heterogeneity, two variants, R14W and E109K, represent more than 50% of the mutational events. To date, treatments for CDAII patients consist of supportive therapy, such as erythrocyte transfusions, or bone marrow transplantation or splenectomy in transfusion-dependent cases. Recently, members of TGF-β superfamily have been studied as potential regulators of erythropoiesis, especially the growth differentiation factor 11 (GDF11). Through the binding of specific receptors, GDF11 leads to an inhibited late-stage erythropoiesis. Indeed, two GDF11 inhibitors, ACE-011 and ACE-536, have been associated with improvements of haematological parameters. Studies with the mouse counterpart of ACE-011, RAP-011, on mouse model of β-thalassemia showed increased differentiation of erythroid cells, improvement of anemic condition and reduced iron overload in treated mice.

Background: Anemia of inflammation (AI) is one of the most common manifestations of iron deficiency in the patients with inflammatory conditions. AI is responsible for hypoferremia, with consequent iron-restricted erythropoiesis with high level of hepcidin, which stimulate the internalization of ferroportin (FPN) transporter. Therefore, inhibiting hepcidin-mediated FPN degradation can be an important strategy to ameliorate AI.

Aims: To increase iron bioavailability we selected a Novel compound against hepcidin action through natural compound libraries that might provide a new alternative approach to increase iron absorption for prevention of hepcidin-mediated FPN internalization and to ameliorate turbine-induced anemic state with different insilico, invitro and invivo studies.

Methods: To find a systemic approach involving in silico, in vitro and in vivo studies was employed to identify hepcidin inhibiting agents. To identify a potent hepcidin-binding agent, natural compounds were screened using molecular docking and dynamics simulations and further investigated on cell lines (GFP-FPN, Caco-2, HepG2) using flow cytometry and western blotting. Normal or turpenline induced anemic mice were used in the associated studies.

Results: The virtual screening via molecular modelling showed that GDP as a potent hepcidin-binding agent as shown in the (Figure 1A). In vitro studies revealed that GDP significantly increased ferroportin stabilization in GFP-FPN cell lines (Figure 1C) and in vivo results showed that co-administration of GDP and ferrous sulphate (FeSO4) significantly improved the turbine-induced anemic state with increase in haemoglobin level (Figure 1B).

Figure 1.

Summary/Conclusions: AI is a normocytic anemia, common among patients with chronic infection and inflammatory disorders. We found that GDP molecule have higher contribution to the stability of hepcidin-GDP complex and thus blocks its interaction with FPN. The results support the novel hypothesis that GDP along with iron supplement regimen can overcome the binding of hepcidin from interaction with FPN that would be an effective treatment for AI.
Aims: The main aim of our study is to assess the effects of RAP-011 on different cellular mediators of ESA response. Methods: We measured circulating GDF11 levels in CD34+ cells 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 silencerfied SEC23B by Sh-CTR and (ii) K562 cells stably overexpressing SEC23B and the two variants, R14W and E109K. In vitro treatment has been performed at 0, 3, and 6 days of erythroid differentiation by hemin+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 to 36% (p=0.02). Stable silencing of SEC23B in K562 cells led to the establishment of two different clones, Sh-70 and Sh-74, showing amarkeddecrease of SEC23Bexpression compared to Sh-CTR (85-90% and 60-65%, respectively). At 3 and 6 days of K562 erythroid differentiation by hemin, we observed an increase of pSMAD2 in GDF11-treated cells compared to non-treated clones; interestingly, a reduction of pSMAD2 in RAP-011+GDF11-treated cells was observed.

Summary/Conclusions: We firstly demonstrated the increased levels of GDF11 in CD34+ cells, which is a more relevant marker of ESA’s effect on the production of red blood cells within the bone marrow and have shown to have a high level of sensitivity (AUC=0.87). We also confirmed the previous results obtained by the in vivo 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 Ha levels in anaemic patients. Uncertainties remain regarding the effect of iron supplementation on the fatal consequences of ESA-treatment.

Aims: The aims of this systematic review and network meta-analysis are to evaluate the effects of iron on 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 anaemia related as well as therapy induced anaemia in cancer patients.

Results: We identified a total number of 105 eligible studies, including 25.722 extracted data and assessed quality of trials. The primary outcome was on-therapy mortality and risk for 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.

Summary/Conclusions: While our analyses show that ESA use increases mortality and risk for 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.
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-THALASSEMIA IN PATIENTS WITH NON-β0/β0 GENOTYPES: THE NORTHSTAR-2 (HGB-207) TRIAL


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Background: Standard treatment for transfusion-dependent β-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 1/2 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 HbA1C (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 4500 [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 HbA1C over time.

Results: As of March 1, 2017, two 20-year-old females with β0/β0 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
by the integration of 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 leukaemia can evade or overcome NK cell detection and killing.

Methods: We used a multidisciplinary approach including RNAseq, Mass Spectrometry, functional 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. Cis was rapidly induced in NK cells in response to IL-15, and deletion of Cis 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 Cis was deleted. Correspondingly, Cis interacted with the tyrosine kinase JAK1, inhibiting its enzymatic activity and resulting in a profound functional ablation. Cis−/− mice are resistant to leukaemia 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 anti-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, we optimized the therapy by using memory stem T cells (TSCM) to promote engraftment and persistence of CD30-CAR T cells after transfer, and we have included an EGFFr depletion 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 in a CD30+ T-cell lymphoma model.

Methods: A second generation CD30-41BBz-EGFRz 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). Naive T cells from healthy donors were activated with anti-CD3/CD28 beads in presence of IL-7, IL-15 and IL-21 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 (IFNγ 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% 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 CAR did not compromise their ex vivo expansion (CD4+ CD30-CAR TSCM: 96.0 ± 3.2 fold expansion; CD8+ CD30-CAR TSCM: 109.0 ± 4.2 fold expansion). CD30+ CD30-CAR TSCM conferred specific cytolic activity and lysed Karpas 299 cells (tumor cell death 1:1 ratio: 92.6 ± 2.4% vs. 0.0% with untransduced TSCM <p>0.001), while control CD30+ target cells (Raji) were not recognized. In addition, CD30-CAR TSCM secreted IFNγ and IL-2 after stimulation with Karpas 299 cells (IFNγ: 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 vitro. 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 cell (MSC) has 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 when an improvement of at least 50% in at least one organ affected by aGvHD was observed, or b) Non-Responders if they had stable or progressive disease.

Results: Patient characteristics are summarized in Table 1.

Table 1.

| Table 1 |
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-mediated 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).

**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 AN NOVEL IRE1 RNASE INHIBITOR MCK-8866 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 cells has been extensively studied, proving the importance of cytoplasmic and nuclear events promoting cell survival. However, a therapeutic strategy involving UPR inhibition has not been considered as a viable option. We identified a strategy that may lead to the development of a novel drug for BCR-ABL+ ALL.

Aims: In this study we aim to identify a potential synergistic effect of simultaneous pharmacological inhibition of IRE1 and BCR-ABL in pre-B cells.

Methods: To study the link between IRE1-XBP1 axis of UPR and BCR-ABL, we utilized both pharmacological and genetic approaches. 1) We tested the effect on proliferation and viability of pharmacological IRE1 inhibition (using MCK-8866) alone and in combination with Tyrosine Kinase Inhibitors (TKI) using Imatinib or Nilotinib on BCR-ABL+ human ALL cell lines, SUP-B15 and TOM-1. The cell lines were also co-cultured with immortalized tertMSCs 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 MCK-8866 (MCK) in combination with either Imatinib (IM) or Nilotinib (NL) showed enhanced capacity to arrest proliferation and to induce cell death in both BCR-ABL+ ALL cell lines compared to single treatments, after 3 days incubation (Viable SUP-B15: MCK 50 µM 94.9±0.1%, IM 10 µM 78.4±0.4%, Combination 17.0±1.4%; MCK 30 µM 94.1±0.7%, NL 5 µM 64.2±0.6%, Combination 20.0±0.8%. TOM-1: MCK 30 µM 85.0±0.9%, IM 10 µM 89.9±0.4%, Combination 17.6±0.7%. MCK 30 µM 94.6±0.1%, NL 5 µM 71.0±0.9%, Combination 30.6±3.6%). Using RNA sequencing for both genotypes and genotypes, 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, present during pharmacological inhibition. After transductions with BCR-ABL1, and either cre or the empty vector, we could observe that heterozygous deletion of Xbp1, induced by 4OHT, significantly increased TKI-induced cell death, after 3 days incubation (4OHT 1µM: 47.5%±13.0%, IM 1µM: 70.8±1.7%, IM+4OHT: 18.3±2.7%, NL 0.5µM: 65.2±0.3%, IM+4OHT: 14.5±0.9%). Finally, we showed whether the tested drugs combination were still effective in presence of BMSCs. It’s known that BMSCs are a critical component to escape TKI-induced cell death in Ph+ leukemia and that IRE1-XBP1 is responsible for chemoresistance in many different cancer types, although this role has never been confirmed in BCR-ABL+ cells. To shed light on this aspect we co-cultured either SUP-B15 or TOM-1 cells with tertMSCs, and while the stroma was capable to block Nilotinib-induced cell death, after 5 days incubation (in SUP-B15, NL 5 µM in standard culture 28.7±1.9%, NL 5 µM in co-culture 74.9±0.1%; in TOM-1, 29.1±2.8 vs 78.7±0.2%), 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. CNAs in pre-B and pre-T ALL, respectively, followed by CDKN2A/B, PAX5, RB1, VPREB1, MLT13, CD200/BLA, TBL1XR1, IKZF1, CASP8AP2, PTPN, RUNX1, BTG1, TP53, IKB2, EZH2, NF1, NR3C2, RAG2 and the PAR region were analyzed for copy number changes. Results were compared to conventional MLPA, cytogenetic and FISH data.

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 patients, including: TP53 (17:1), BCR-ABL1 (19:0), TP63 (4:0), CASP8AP2 (5:0), IKZF2 (1:0), CDKN2A (3:0), CDKN2B (2:0), PTPN11 (2:0), IKB2 (1:0), TP53 (1:0), RB1 (1:0), PTEN (1:0), MLLT3 (1:0), TBL1XR1 (1:0), IKB2 (1:0), and IKB2 (1:0). Overall, our data demonstrate that simultaneous inhibition of both BCR-ABL1 and IRE1 significantly improves cellular efficacy and confidence as compared to conventional MLPA and enabled a more patient-specific characterization of CNAs, as revealed by 15 different deletion patterns across 23 samples harboring del(6p). In addition to genomic lesions specifically influencing putative or proven driver or relevant genes, 24 structural (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 in 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(6p). In addition to genomic lesions specifically influencing putative or proven driver or relevant genes, 24 structural (134 whole chromosome aberrations were detected genome-wide which was strongly facilitated by the inclusion of ~200 digital karyotyping probes covering each chromosome arm.

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 the pathogenesis of B-ALL is still a matter of debate.

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 (Dex), and Notch modulators including anti-Notch blocking antibodies, gamma secretase inhibitors (GSIs), and Notch transcription factor inhibitor (SAHM1). Mouse xenograft model of B-ALL were obtained by injecting the B-ALL line RS4;11 in
NOD/Shi-scid/lL-2Rnull 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 Doxo up-regulated 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 Doxo towards B-ALL cells. Finally, we evaluated the administration to mice of a pan Notch inhibitor, i.e. the GSI XII, significantly lowered the CD19+ leukaemic 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 mir-181ab1 blocks Notch-induced T-ALL development partly by de-repressing the expression of NRARP (NOTCH regulated ankyrin repeat protein) a negative regulator of NOTCH signaling. Importantly, NRARP overexpression in human hematopoietic stem cells impairs T-cell development suggesting that de-regulation 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 normal 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 oncogenic 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 QOJ, a small-molecule bromodomain inhibitor that targets c-Myc. NRARP is known to positively regulate LEPF, a DNA binding transcription factor acting downstream of WNT. Thus we sought to investigate the impact of NRARP on NOTCH1 signaling pathway. Very interestingly, our results show that in NOTCH1-mutant cells NRARP overexpression results in the down-regulation of the WNT signaling 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 belong to the recently described ETV6/RUNX1-like ALL (Liljebjörn et al., Nature Communications 2016).

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/CD44low B-other cases that were classified as ETV6/RUNX1-like ALL. We identified multiple regions of acquired copy number aberrations (CNA) unparental disomies (5 to 27 per case) and point mutations (10 to 41 per case) in all 7 cases and 3 in-frame fusions transcribed each in one patient. The most important findings are summarized in Figure 1. All 5 ETV6/RUNX1-like cases harbored a deletion of the ETV6 gene, resulting in an in-frame ETV6/BORC5 fusion in one of them. The deletion of ARP2P21 was found in 3 cases, and the deletions of PAX5, ATP10A, BTG1 and the gain of RUNX1 were found in 2 cases each. The ARP2P21 deletions displayed a strikingly uniform character and were highly enriched in ETV6/RUNX1-like ALL. Using WES and RNAseq, no recurrently mutated gene and no in-frame fusions were found, respectively, except for the ETV6/BORC5. Integrating data from all platforms, we identified IKZF1 as another recurrently affected gene; a deletion, a nonsense mutation and an IKZF1-involving out-of-frame fusion were found in one case. The other cases with available material. 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/CD44low B-other cases clustered within the ETV6/RUNX1-positive cluster. These B-other cases were thus classified as ETV6/RUNX1-like ALL. We identified multiple regions of acquired copy number aberrations (CNA) unparental disomies (5 to 27 per case) and point mutations (10 to 41 per case) in all 7 cases and 3 in-frame fusions transcribed each in one patient. The most important findings are summarized in Figure 1. All 5 ETV6/RUNX1-like cases harbored a deletion of the ETV6 gene, resulting in an in-frame ETV6/BORC5 fusion in one of them. The deletion of ARP2P21 was found in 3 cases, and the deletions of PAX5, ATP10A, BTG1 and the gain of RUNX1 were found in 2 cases each. The ARP2P21 deletions displayed a strikingly uniform character and were highly enriched in ETV6/RUNX1-like ALL. Using WES and RNAseq, no recurrently mutated gene and no in-frame fusions were found, respectively, except for the ETV6/BORC5. Integrating data from all platforms, we identified IKZF1 as another recurrently affected gene; a deletion, a nonsense mutation and an IKZF1-involving out-of-frame fusion were found in one case. The other cases with available material. 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).

Summary/Conclusions: We showed that similarly to ETV6/RUNX1-positive ALL, ETV6/RUNX1-like ALL is also associated with CD27pos/CD44low/neg immunophenotype. We identified deletion of ARP2P21 to contribute to the specific genomic profile of ETV6/RUNX1-like ALL in addition to lesions of ETV6

Figure 1.
and KZF1. In conjunction with the single published study, our study establishes the ETV6 lesion as the only common genetic aberration and thus the most likely key driver of ETV6/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 as well as their impact on outcomes in those treated with TPOAG-2002 protocol. Methods: Between 1995 and 2015, bone marrow samples from 102 children (<18 years old) consecutively diagnosed with T-ALL were examined. SIL-TAL1, MLL-ENL, and CALM-AF1/20 transcripts were detected by RT-PCR assays. RTPCR with TaqMan assays were used to measure the expression of HOX11, TAL1, and NYLI oncogenes expressed as normalized copy number (NCT) to ABL internal control gene. TAL1 overexpression was defined as NCT > the lowest level of SIL-TAL1 positive patients. Overexpression of HOX11 and TAL1 was defined as NCT > the upper limit of the 95% confidence interval control genes. 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 mutations, MYB duplication and NUP214-ABL1 deletion. Results: The frequency of SIL-TAL1 fusion transcript was 16.2%, MLL-rearranged 5.1%, CALM-AF1/20 1.0%, and no NUP214-ABL1. The frequency of NOTCH1 mutations was 46.9%, FBXW7 13.0%, RUNX1 5.2%, JAK1 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 26.4%. 62% of patients were positive for HOX11. The correlation among the genetic alterations showed that NYLI overexpression occurred more frequently in P16 wild-type compared with P16 deleted patients (P<0.003) and absence of SIL-TAL1 transcript was significantly associated with NYLI overexpression (P<0.018). A comparison of outcomes was made according to the status 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) (P=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 NYLI overexpression had no prognostic impact. Multivariable analysis, NCT1 muta-tions, and clinical characteristics achieved significant influence for an independent predictor of OS (HR 0.167, P=0.112), PHF6 deletion/mutation was an independent unfavorable predictor for OS (HR 4.596, P=0.006), and PTEN deletion was 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 9.639, P=0.014) and OS (HR 2.701, P=0.047).

Summary/Conclusions: The present study showed that NYLI overexpression was negatively associated with SIL-TAL1 or P16 deletion. PHF6 deletion/mutation, PTEN deletion, and TAL1 overexpression were the independent predictors of adverse outcomes. (Grants support: CORPG3C0201, MH-E-105-09, NSC-101-2314-B-195-004-MY2, and Tyre Fox 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 chromatin insufficiency of a neighboring 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 applying pattern recognition techniques to a copy-number dataset. This approach will identify genomic regions with potential essential genes. Genes in these regions can be evaluated downstream by genome editing techniques to find novel targets for treatments. Using pattern recognition techniques to find essential genes is straightforward, 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 visualisation and by investigation of their known function. The framework we 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 potential 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 cell-lines.

E827
TARGETING ANTIOXIDANT ENZYMES FOR THE TREATMENT OF B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: B-cell acute lymphoblastic leukemia (B-ALL) is a genetically heterogeneous disease characterized by abnormal expansion of B cell precursors and is mainly affecting children and adolescents. The backbone of the treatment is chemotherapy providing high cure rates in pediatric ALL (> 85%) but much worse treatment response and even lethal outcome in adults (ponse 50%). Patients who relapse develop refractory, chemotherapy resistant disease and remain a clinical challenge. Growing body of evidence suggests that disturbance of redox homeostasis is a promising anticancer approach. Due to high metabolic demands and proliferation rate cancer cells elevate their antioxidative capacity to overcome excessive ROS production and depend on these antioxidant interventions for their survival. One of the oxidative stress protectors are peroxiredoxins (PRDXs) that next to thioredoxins (TXNs) belong to the TXN-family and are the key components of TXN antioxidant system. PRDXs are enzymes involved in scavenging peroxides. TXNs are responsible for cysteine-thiol disulfide exchange in numerous protein substrates.

Aims: To investigate the potential of targeting the TXN antioxidant enzymes as a novel pro-oxidative strategy in B-ALL treatment.

Methods: We have used three different cell lines representing distinct cytogenic subgroups of B-ALL: BV-173 (BCR-ABL), SEMK-2 (MLL-AF4) and NALM-6 (t(12;21)). ROS levels were measured using Quant-ROS, RNA and protein levels of TXN-family enzymes were measured by quantitative PCR and immunoblotting, respectively. Downregulation of PRDX1 was established by a novel CRISPR/Cas9 gene editing system. We have employed lenti-
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), auranothin (AUR) and SK053 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 ETV6/RUNX1 LEUKEMIA CELLS

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Background: The IGF2 mRNA binding protein 1 (IGF2BP1, other aliases IMP-1 (IMP1), CRD-BP (CRDBP), 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 ETV6/RUNX1-positive acute lymphoblastic leukemia (ALL) but biological significance of IGF2BP1 overexpression has not been thoroughly investigated to date (Andersson, Olofsson et al. 2005; Stokšus, Gineikiene et al. 2011). We have recently contributed by reporting that ETV6/RUNX1 transcript is a target of RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL, suggesting a role of IGF2BP1 in ETV6/RUNX1-mediated leukemogenic events (Stokšus, Vaitkevičienė et al. 2016).

Aims: To define the biological significance of IGF2BP1 overexpression in t(12;21)(p13;q22) ETV6/RUNX1-positive ALL.

Methods: In this study we have used stable sublines with downregulated IGF2BP1 from our previously published study (Stokšus, Vaitkevičiene 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, ETV6/RUNX1, and STAT3 RT-qPCR was performed previously (Stokšus, 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 ETV6/RUNX1 mRNA (r2=0.8253, p<0.001, slope 0.9459) and also STAT3 transcript levels (r2=0.7709, p=0.002, slope 0.6436). 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 ETV6/RUNX1®STAT3 signaling axis is one of the possible mechanisms responsible for this phenotype as IGF2BP1 maintains appropriate levels of primarily ETV6/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 (Stokšus, 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 phosphatidylinositol 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 cell proliferation 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 (SM) on the remitting T-cell leukemia cells metabolic reprogramming.

**Methods:** In vitro experiments were performed in a Jurkat T cell line. Cells were incubated with 6-MP from 6h to 72h. We used RT-PCR, Western Blot, glucose uptake and glycolytic and glutaminolytic flux to evaluate the metabolic effects of 6-MP.

**Results:** Our results showed that 6-MP reduces ATP content as early as after 2 hours of treatment and this decrease is maintained up to 72 hours. As AMPK is an energetic sensor activated with low ATP content, we studied AMPK activation after 6-MP treatment. We observed that 6-MP treatment activates AMPK after 6 and 48 hours of treatment. Moreover, 6-MP significantly modifies the transcriptional activity of hypoxia inducible factor 1α. Genes implicated in glycolysis, glutaminolysis 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). In addition, our original approach to better understand the cellular effects of 6-MP treatment.

**Conclusion:** In conclusion, our findings offer new insights on the cellular effects of 6-MP treatment by promoting an early energetic stress that influence 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.

**E830**

**GENETIC ABERRATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA AND THEIR IMPACT ON CLINICAL OUTCOME**

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**Background:** Genetic alterations have prognostic impact on pediatric patients with B cell acute lymphoblastic leukemia (B-ALL). Genomic landscape and its impact on clinical outcome is less understood in adults with B-ALL.

**Aims:** To describe the landscape of genomic aberrations and analyze the correlation with clinical characteristics and prognostic impact in adults with B-ALL.

**Methods:** We assessed bone marrow specimens from 64 consecutive adults with a median age of 51 years (range 18 to 80) with previously untreated B-ALL between 2012 and 2015. The cohort included 23 Philadelphia chromosome (ph)-positive and 41 Ph-negative B-ALL. Sixty patients (94%) were treated with Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone) and prednisolone (prednisone 16 mg/m², dexamethasone alternating with high-dose methotrexate and cytarabine)-based regimen; 23 of them received additional tyrosine kinase inhibitor for Ph-positive disease (dasatinib [N=4] or pontinib [N=19]). Four patients (6%) were treated with augmented BFM (Berlin-Frankfurt-Munster) regimen. Genomic DNA extracts were sequenced by targeted capture exome sequencing of 295 genes that are recurrently mutated in hematologic malignancies (median 280x coverage). The panel comprised a minimum of 25 markers, and 25 markers and 320Mb for CN-LOH.

**Results:** Among the 64 patients, we detected 70 point mutations in 40 genes in 38 patients (54%). Ph-positive ALL had significantly less point mutations than Ph-negative ALL (median number of mutations/patient 0 [range: 0-2] versus 1 [range: 0-4]; P=0.002). The most frequently mutated genes were TPS5 (17%) followed by MLLT3 (10%), AK2 (6%), NRAS (6%), NFI (5%), RUNX1 (5%), and TET2 (5%). TP53 mutations were strongly associated with Ph-negative B-ALL (P=0.004) and low hypothyroidism (P=0.009). Recurrent CNVs involved loss/deletion in genes such as PAX5 (38%), TCF3 (38%), IKZF1 (31%), CDKN2A/2B (31%), BTLA (25%), CD200 (22%), ETV6 (22%), RBL (20%), MIR15a/16-1 (15%), EG14 (14%), and MLLT3 (11%), whereas gain/amplification was detected in NRASC2 (18%), ERG (15%), RUNX1 (15%), and LEF1 (14%). MLLT3 loss/deletion was specific to Ph-negative ALL (0% for Ph-positive versus 17% for Ph-negative, P=0.036) and MIR15a/16-1 loss/deletion had non-statistically significant association with Ph- ALL (4% versus 22% respectively, P=0.08). In this cohort, 78% and 100% of the Ph-negative and Ph-positive ALL achieved complete remission, respectively. None of the point mutations or CNVs were associated with differential response to therapy. Survival analysis was stratified by Ph status. Complex karyotype had trend toward worse event-free survival (EFS) (median EFS 3.6 months versus 26.3 months, P=0.06) in Ph-negative ALL. None of the point mutations or CNVs were associated with EFS/overall survival (OS) in Ph-negative ALL. Notably, TP53 mutation nor low hypothyroidism did not affect EFS/OS in the current cohort. In Ph-positive ALL, IKZF1 deletion/loss was associated with a trend toward worse EFS (median EFS 3.6 months versus 21.3 months, P=0.07) but it did not affect OS.

**Summary/Conclusions:** Genetic analysis highlights the molecular heterogeneity of adult B-ALL. Adult B-ALL is frequently associated with CNVs and point mutations are less frequent. Prognostic impact of genetic alteration in adult B-ALL appears to be limited except for IKZF1 deletion/loss, which may predict worse EFS in Ph-positive B-ALL.

**E831**

**PROFILING OF RECURRENT COPY NUMBER ALTERATIONS IN RELAPSED ADULT B CELL PRINCIPAL 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 (BCP-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). CNV analysis was performed for all genes and encompassed a minimum of 25 markers, and 25 markers and 320Mb for CN-LOH.

**Table 1:**
Results: With a median follow up of 12.43 [2.4;3.0] months, the median OS of the 31 patients at first relapse was 7.9 months. [2.4;13.5]. The OS of patients at first relapse was significantly lower in those having more than 3 CNA by MLPA (median s3 CNA 9.7 months [0-20.9] vs median s3 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%). ORR of patients harboring CDKN2A/B deletions was 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 (9.6 DEL, 2.3 DUP and 0.4 LOH); while at 2nd 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 0.4 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 different biological rationale in their acquisition). Finally, the most retained or acquired CNA at diagnosis were 9p, 11q, 12p, 22q and 7p deletions and 1q, 8q, 17q, 21+ and 20+ duplications. The 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 treatments depending on their molecular features. Finally, we propose that the Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, Spain, Red Temática de Investigación Cooperativa en Cáncer (RTICC, FEDER) (RD12/0036/0044); Sociedad Española Hematología y Hemoterapia; 2014 SGR226 (GRE) Generalitat de Catalunya; Fundació Internacional Josep Carreras, Celgene Spain and “la Caixa” Foundation.

E832

IGF1/IRS PHARMACOLOGICAL INHIBITION REDUCES CELL PROLIFERATION AND MIGRATION IN ACUTE LYMPHOBластIC LEUKEMIA CELLS

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Background: A recurrent clinical complication of the acute lymphoblastic leukemia (ALL) is the infiltration of lymphoblast into central nervous system. The IGF1/IGFR signaling pathway is initiated through binding of the ligand (IGF1) to its transmembrane receptor (IGFR1), and the subsequent activation of its substrates, IRS1 and IRS2, which transmit mitogenic and antiapoptotic signals, mainly through the modulation of the PI3K/AKT/mTOR and MAPK signaling pathways. These signaling pathways play an important function in cell proliferation, survival and migration of leukemia cells. We have previously noticed that NT157 (IRS1/2 pharmacological inhibitor) significantly decreased cell viability and induced apoptosis in T-ALL (Jurkat and MOLT-4), and in B-ALL (Namalwa and Raji) cell lines and in primary ALL cells (T-ALL [n=2] and B-ALL [n=2]), although did not presented citotoxicity in peripheral blood mononuclear cells (PBMC) from healthy donors. In addition, NT157 was able to induce the p21 (CDKN1A) expression, which is a cell cycle arrest-related gene. We also observed that OSI-906 (IGF1/IGFR pharmacological inhibitor) significantly reduced cell viability, but did not induce apoptosis in ALL cell lines tested, and did not modulate viability and apoptosis of primary ALL cells and normal PBMC. The molecular mechanism by which leukemia cells break the blood-brain barrier, allowing the infiltration of the central nervous system and causing serious complications is not well understood.

Aims: We herein aimed to investigate the impact of the pharmacological IGF1/IR and IRS1/2 inhibition on cell proliferation and migration in ALL cells.

Methods: T-ALL Jurkat and B-ALL Namalwa were used. Cell lines were treated or not with NT157 at 0.2, 0.4, 0.8, 1.6 and 3.2 µM, or with OSI-906 at 0.5, 5, 10, 20 and 40 µM for 24 and 48 hours. After drug exposure, cell lines were evaluated for cell proliferation (Ki67 assay), migration (Time-Lapse microscopy analysis) and cell adhesion (using human umbilical vein endothelial cells HUVEC monoclonal). Statistical analyses were performed by the ANOVA. Value p <0.05 was considered statistically significant.

Results: In ALL cells, NT157 strongly reduces cell proliferation in a dose-dependent manner (p<0.05) after 24 hours of treatment. OSI-906 was not able to reduce cell proliferation in these cell lines. The 24 hours treatment with 10µM OSI-906 decreased accumulated distance (µm) and velocity (µm/min), while 0.4µM NT157 reduces only the accumulated distance of Jurkat cells under migration assay into fibronectin monolayer, after being filmed by time-lapse microscopy for 3 hours; the images were captured every 1.5 minutes. Although there is a trend for reduction, cell adhesion between Jurkat and Namalwa leukemia cells and the human endothelial cell monolayer was not significantly modulated by treatment with both inhibitors.

Summary/Conclusions: The reduction in cell proliferation found during IGF1/IRS pharmacological inhibition reaffirms the important role of these proteins on malignant phenotype of ALL cells. Migration analysis indicated that NT157 and OSI-906 are potential inhibitors of transendothelial migration in ALL cell lines and contribute with new perspectives on the participation of the IGF1/IRS1 pathway in the break of the blood-brain barrier.

E833

LEUKEMIA-PROVAGATING CELLS DEMONSTRATED DISTINCTIVE GENE EXPRESSION PROFILES COMPARED WITH THE OTHER CELL FRACTIONS IN PATIENTS WITH DE NOVO PHILADELPHIA CHROMOSOME-POSITIVE PH+ALL

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Background: Relapse remains one of the major obstacles in Philadelphia chmosome-positive acute lymphoblastic leukemia (Ph+ALL) even after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Relapse of Ph+ALL may be associated with the persistence of leukemia propagating 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 outcome. Here, we aimed to explore how relapse LPCs were 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 2018. The LPCs (CD34+CD38−CD58−) and other cell fractions (including CD34+CD38+CD58+, 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 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 Cell1 (patient No 1), and Other Cell3 (patient No 2), and LPCs, and Other Cell2, (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 process, ribonucleotide binding process, nucleoside binding process, DNA replication process, 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 H2AX, 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 between LPCs and the other cell fractions in patients with de novo Ph+ALL. 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.
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 LYMPHOBластIC 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 region-specific hospitalization days per patient in the INO-VATE trial.

Methods: Patients receiving study treatment (safety population) and recruited from the US and 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.

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-INTERMITTENT BUT NON-INTERRUPTIVE TREATMENT WITH FEWER ALLO-HSCT IS EFFECTIVE STRATEGY FOR ADULT PH-NEGATIVE B-CELL PRECURSOR (BCP-) 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 portion of allo-HSCT, the results of treatment based on the different approaches are escalating 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.

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.
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 (m.age 28 y (15-55), f/m 147/182) were recruited. Phenotype was unknown in 6 pts, biophenotypic AL was diagnosed in 1 (2%), T-ALL/LBL in 36.7% (n=125), BCP-ALL in 51.9% (n=194). Among BCP-ALL there were 54 early pre-B ALL (72.8%), 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 >9140 FMO (0.4-8990), LDH 901 IU (31-13059), CNS leukemia in 17 pts (8.7%), mediastinal mass in 3 (1.5%), splenomegaly in 111 (57.2%). Standard cyto- genetics was documented in 124 pts (64%), 11% had no mitosis, so information is avail- able in 58.2% (n=112). 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 (16%), del(1p) (22.2%, etc. 9 BCP- ALL patients (n=47) were not qualified by the risk in the data-base: 68.1% (n=126) were attributed to the high risk (HR) group (WBC >30; EGG, 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 (7.9% (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 1CR. Totally 59 pts (34.9%) had relapsed. At 7y OS for the whole cohort consti- tuted – 54,3%, DFS – 56.5%, RP – 35,4%. In a multivariate analysis for BCP- ALL HR group (age >27y, WBC>75*109/l, t(4;11)).

Summary/Conclusions: Our data demonstrate that non-intensive but non- interruptive treatment with fewer allo-HSCTs is rather effective in adult BCP- ALL producing more than 50% OS at 7 years, though the RP is high. In our study among common risk factors only age, initial WBC and t(4;11) remained the most valuable markers of poorer prognosis, while immunophenotype, time to CR, CNS involvement, and other cyto- genetic markers did not matter. So RALL protocol without intensive highly myelosuppressive consolidation courses and high portion of autologous HSCT, may become an alternative and reproducible approach for adult Ph-negative ALL.

E837

POST-INDUCTION MINIMAL RESIDUAL DISEASE RESPONSE DETERMINED BY MULTICOLOR FLOW CYTOMETRY IS A POWERFUL INDICATOR OF EVENT-FREE-SURVIVAL IN THE CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Minimal residual disease (MRD) is a powerful predictor of event- free survival in acute leukemia including T-cell acute lymphoblastic leukemia (T-ALL). Due to lower incidence of T-ALL MRD studies are limited and restricted to a small cohort of patients. Moreover, flowcytometry based MRD (FC-MRD) studies in T-ALL are very few. AIEOP-BFM group showed that late (Day-78) MRD response determines overall risk-of-relapse and event-free survival (EFS) using RQ-PCR. However, a larger study by COG (Brent Wood et al, 2016) demonstrated 7y OS=79%, DFS=71%, RP=23% in the standard risk (SR) group (age >27y, WBC >75*109/l, 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).

Methods: We studied post-induction (Day-35) MRD (PI-MRD) & post-consol- idation (Day-78) MRD (PC-MRD) in bone marrow samples from 100 patients of T-ALL treated under modified MCP-841 protocol between 2014 & 2016. In T-ALL with early-thymic-precursor (ETP) immunophenotype, patients received dexamethasone in place of prednisolone. MRD was performed using 10-color FC-MRD assay on Navios flow-cytometer (Beckman Coulter, BC) and MRD analysis was performed with Kaluza software v-1.3 (BC). Any detectable level of MRD (≥20 events) was defined as MRD-positive. Events included relapse & disease-related deaths. Statistical analysis was performed using SPSS v.16.

Results: The median age of patients was 11.5 years (range 2–16 y; M:4–6). Based on the immunophenotypic criteria, 13 patients were diagnosed as ETPLL & remaining 87 as non-ETPLL type. PI-MRD was positive in 58/100 (58%) with the median level of 0.23% (range, 0.002% to 6%). PC-MRD was not performed in 71.4% (30/42) of Ph-negative & 1.2% (6/58) Ph-MRD- positive patients. PC-MRD was available in 64 patients (30/42 of PI-MRD-neg- ative & 6/58 of PI-MRD-positive). PC-MRD was positive in 28% (18/64) (median, 0.2% & range, 0.009% to 4%). PI-MRD positivity was significantly high in ETPLL as compared to non-ETPLL (93% vs 53%; p=0.01). Median follow-up of all patients was 14 months (3-38 months). Patients were categorized MRD standard-risk (MRD-SR) if PI-MRD was negative and MRD high-risk (MRD-HR) if PI-MRD was positive with any level. Thus, 42% were categorized as MRD-SR & 58% as MRD-HR. Twenty patients relapsed & of them, six died (2 were ETPLL & 18 non-ETPLL; 3 MRDSR & 17 MRD-HR) within 26 months. Median EFS of MRD-HR patients was significantly inferior as compared to MRD-SR (26 months vs did not reach; & 70.67% vs 92.86%; p=0.0017) (Kaplan-Mayer curve shown in Figure 1). Interestingly, there was no difference in EFS for PI-MRD <0.01% vs >0.01%, suggesting any level of PI-MRD positive indicated inferior EFS. Furthermore, the PC-MRD response was not found to be significant over PI-MRD (P-value=0.17). ETP vs non-ETP status was also not found to be associated with EFS (P-value=0.85).

Figure 1.

Summary/Conclusions: We concluded that 10-color FC-based post-induction MRD response is a powerful indicator of EFS in childhood T-ALL. The frequency of PI-MRD positivity was significantly high in ETPLL indicating a lower tumor clearance rate. There was no difference in the EFS based on the level of PI- MRD-positivity indicating even a low level (<0.01%) PI-MRD is important in risk-stratification of childhood-TALL.

E838

SMAC MIMETICS - A NOVEL THERAPEUTIC APPROACH IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Pediatric acute lymphoblastic leukemia (ALL) is one of the most common malignancies in childhood. Survival rates have increased enormously over the past decades, but the prognosis for patients with relapsed ALL or ALL after alloHSCT is rather poor. Thus, novel therapeutic options are urgently required. The family of inhibitor of apoptosis proteins (IAPs) has been shown to play an important role in the prevention of cell death, and to mediate gene activation important for cell survival. Many of the cellular processes regulated by IAPs are deregulated in cancer. Thus, IAPs represent a promising target in anticancer therapy. IAP antagonists, also known as SMAC Mimetics (SMs), were developed to counteract IAPs function. SMs have been shown to induce cell death in a number of different cancer entities, amongst them B cell precursor (BCP)-ALL. In BCP-ALL, SM-induced cell death was...
**Background:** Acute lymphoblastic leukemia (ALL) exhibits a bimodal age distribution with 60% of cases occurring in children and adolescents (<20 y) and 25% in older adults (>45 y; http://seer.cancer.gov/csr/1975_2013/). As many as 20% of children relapse after initial therapy, with subsequent poor clinical outcomes (Front Oncol 2014;4:63). Promising results were observed with KTE-C19, an anti-CD19 chimeric antigen receptor (CAR) T cell therapy, in B cell malignancies, including refractory, aggressive non-Hodgkin's lymphoma in the ZUMA-1 trial (Blood 2016;128:LBA-6). Here, we present updated results from the phase 1/2 trial of KTE-C19, a phase 1/2 trial of KTE-C19 in pediatric and adolescent patients with relapsed/refractory relapsed/refractory acute lymphoblastic leukemia.

**Aims:** The aim of the phase 1 study is to evaluate the safety of KTE-C19 in pediatric and adolescent patients with R/R ALL. Methods: Pediatric and adolescent patients (aged 2-21 y) with high burden R/R ALL (>25% marrow blasts), adequate renal, hepatic, pulmonary and cardiac function received 2×10^6 CAR T cells/kg after low-dose conditioning chemotherapy consisting of cyclophosphamide (900mg/m² once) and fludarabine (25mg/m²/d for 3 days) (CyFlu). The primary endpoint is the overall response rate of 1 phase 1 trial. The secondary endpoints include efficacy outcomes and biomarker assessments.

**Results:** As of 19 Jan 2017, 5 patients have enrolled and 4 have been treated with KTE-C19 at 2×10^6 CAR T cell/kg. KTE-C19 was successfully manufactured in a centralized, streamlined 6-8-day process for all patients across a wide range of baseline absolute lymphocyte counts (0.21–1.0×10^9/L), except in 1 patient who had disease progression with white blood cells 150,000/µL at apheresis and <0.2% T cells in the apheresis collection. All 4 treated patients had high disease burden with a median marrow lymphoblast content of 57%.
(range, 41–99%). All 4 patients received bridging chemotherapy during the manufacturing period before conditioning chemotherapy and KTE-C19. No patient experienced a dose-limiting toxicity. One patient had a grade 5 adverse event of disseminated mucormycosis which was not related to KTE-C19. Cytokine release syndrome was reported in all 4 patients (all ≤ grade 3); neurologic events were reported in 1 patient (grade 3). All cytokine release syndrome events resolved with tocilizumab, corticosteroids, and/or nilotinib plus other supportive care with a median duration of 8.5 days (range, 4–16 days). Minimal residual disease-negative remission was observed in all 4 patients. One patient received stem cell transplant post-remission, which is allowed per protocol at investigator discretion. Peak expansion of CAR T cells occurred 1–2 weeks post-KTE-C19 infusion. Updated data with additional patients, different dose of KTE-C19, earlier tocilizumab use, and biomarkers will be presented.

Summary/Conclusions: KTE-C19 after low-dose CyFlu has been tolerable and appears safe for further analysis in pediatric and adolescent patients with R/R ALL. No toxicities were observed with KTE-C19 at the 2×10^6 cells/kg dose in patients despite high leukemic burden. All patients receiving KTE-C19 achieved a minimal residual disease-negative remission. Based on these results, ZUMA-4 continues to enroll (NCT02625480).

**E841**

**COMPARISON OF 8-COLOR FLOW CYTOMETRY AND PCR-BASED METHODS IN MEASUREMENT OF MINIMAL RESIDUAL DISEASE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA**

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Background: The presence of minimal residual disease (MRD) is the most important prognostic factor in adult acute lymphoblastic leukemia (ALL). MRD monitoring is routine performed by flow cytometry (FCM) and real-time quantitative polymerase chain reaction methods (RT-PCR).

Aims: We conducted a retrospective analysis comparing these MRD measurement methods in ALL patients treated in three Czech hematology/oncology centers within the CELL group (Czech Leukemia Study Group for Life).

Methods: Adult patients (age 18-55) with both Ph-negative and positive ALL were enrolled in the study, all treated consecutively between 2008 and 2016 according to a pediatric-inspired CELL ALL protocol. Samples for MRD evaluation were acquired from bone marrow on day 26 of induction (D26) and in the 11th week of treatment before the first consolidation (W11). We divided RT-PCR MRD positive and negative groups using three different cut-off values and analyzed them separately: 1) 1.0×10^{-3}, 2) 1.0×10^{-4}, 3) every RT-PCR positive result considered MRD positive even below 1.0×10^{-4}. Cut-off value 1.0×10^{-3} was used for FCM MRD. Results were statistically analyzed by the Kaplan-Meier method and log-rank (Cox-Mantel) test.

Results: Total number of 103 patients was evaluated. Nine of them (8.7%) did not reach a hematological remission on D26 were excluded from the study. The 5-year event-free survival of the final cohort was 76.7% for FCM (N=73) and RT-PCR of immunoglobulin heavy chain (IGH, 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.

**E842**

**QUALITY-ADJUSTED LIFE YEARS (QALY) FOR INOTUZUMAB 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-calicheamicin 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 over SOC were from Post-HSCT. These gains are greater in the InO arm and potentially curative hematopoietic stem cell transplantation (HSCT), and favoring the 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.

Summary/Conclusions: This analysis taking into account both quantity and quality of life estimates shows that InO offers an average of nearly 2 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.

**Table 1.**

<table>
<thead>
<tr>
<th>Health state</th>
<th>INO</th>
<th>SOC</th>
<th>INO-SOC</th>
<th>QALY</th>
<th>INO-SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CR</td>
<td>0.07</td>
<td>0.13</td>
<td>-0.06</td>
<td>0.63</td>
<td>0.06</td>
</tr>
<tr>
<td>CR</td>
<td>0.25</td>
<td>0.08</td>
<td>0.17</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>Post HSCT</td>
<td>2.98</td>
<td>2.62</td>
<td>0.36</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>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>3.46</td>
<td>1.77</td>
<td>1.69</td>
<td>2.48</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Increment values may not always correspond to differences between LYs and QALYs due to rounding.

**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**

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Background: Minimal residual disease (MRD) detection is important in adult acute lymphoblastic leukemia (ALL), but RQ-PCR is not the assessment method for all patients due to non-applicable RQ-PCR target or applied RQ-PCR criteria.

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: Our analysis has shown 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^{-3}) into account in W11 and later stages of treatment. FCM 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.

Supported by MUNI/A/1106/2016 grant of Masaryk University, Czech Republic and the Czech Leukemia Study Group for Life.
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Background: Minimal residual disease (MRD) has been proven to be the most important indicator of relapse in BC-PALL. Recently, flow-cytometry based MRD has been shown to achieve a sensitivity of <10-5 using a standardised panel with high number of event acquisition. However, high-sensitivity BMRD analysis is based on experience and acquisition of high number of events also includes other rare BM cellular elements and artifacts. We present a study of the cost-effective high-sensitivity 10-color single tube FC-MRD assay in BC-PALL along with description of rare BM cellular elements and artifacts causing interference in analysis.

Aims: 1. To study the applicability and sensitivity of a 10-color high event single tube FC-MRD assay for BC-PALL; 2. To document the rare BM cellular elements and artifacts causing interference in high-sensitivity FC-MRD assay for BC-PALL and describe their immunophenotypic features.

Methods: We studied 230 BC-PALL 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, CD33, CD56, CD98, CD123 and CD25/COD73 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 BC-PALL 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) by performing dilution assay. 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% and >1% and they were respectively 1.74%, 10.43%, 13.48%, 5.65% and 10.00%. Furthermore, in 24 samples with MRD-positive ≥0.1% and >1.5 million acquired-events, the results were compared between time-gated initial 500000-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 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 (BPC); 6) CD19+ NK cells (Table 1). We also described their immunophenotypic features highlighting the differentiating features from MRD and B cell precursors (Figure 1).

Table 1.

<table>
<thead>
<tr>
<th>Feature</th>
<th>CD34</th>
<th>CD10</th>
<th>CD73</th>
<th>CD123</th>
<th>CD86</th>
<th>CD58</th>
<th>MNC</th>
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<tr>
<td>B cells</td>
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<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
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<tr>
<td>BPC cells</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
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<tr>
<td>NK cells</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
</tbody>
</table>

Summary/Conclusions: We established a cost-effective 10 color single tube FC-MRD assay with high sensitivity of least 1 in 105 and applicability in >97% BC-PALL MRD samples. We also described the frequency and extent of different cellular events and artifacts that can interfere with high-sensitivity BC-PALL 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.

E844

SPECKLE TRACKING ECHOCARDIOGRAPHY IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: A PRELIMINARY STUDY

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Background: Children with acute lymphoblastic leukemia (ALL) are at risk for late-onset anthracycline-related cardiotoxicity. Left ventricular Global Longitudinal Strain (GLS) by Speckle Tracking Echocardiography has been recently used to identify preclinical late-onset heart failure in both adult and pediatric ALL patients. Nonetheless, efficient strategies for the early diagnosis and management of cardiac toxicity during chemotherapy are still not defined.

Aims: We prospectively studied LV function in ALL patients treated at our Centre, according to AIEOP-BMF ALL 2009 protocol, measuring both GLS and M-Mode Left Ventricular Ejection Fraction (LVEF).

Methods: Out of 42 consecutively examined ALL patients (pts), 32 (76%) underwent prospective follow-up (Table). At basal evaluation, 19 pts had Hb<9 g/dl, 7 pts had fever, 7 pts had hyperleukocytosis, 2 had BMI>25. The influence of these factors on cardiovascular parameters could not be evaluated due to low numbers. Echocardiography was performed with Vivid E9 ultrasound system (GE Medical System) and M5Sc probes at the following time points (TP): diagnosis (TP0), after induction phase (anthracycline cumulative dose 120mg/m2, TP120), at the end of anthracycline exposure (TP240 in standard risk-SR-pts; TP320 in high risk-HR-pts). HR pts underwent intermediate controls before each re-exposure (TP150, TP210, TP270). GLS values>−0.1% (or GLS drop between basal and LVEF value) and LVEF values<55% (or LVEF drop >10% from basal value) were considered as abnormal. Statistical analysis was performed using Student's t-test.

Table 1.

<table>
<thead>
<tr>
<th>TP</th>
<th>GLS</th>
<th>LVEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP0</td>
<td>3 (11%)</td>
<td>50%</td>
</tr>
<tr>
<td>TP120</td>
<td>0 (0%)</td>
<td>55%</td>
</tr>
<tr>
<td>TP240</td>
<td>0 (0%)</td>
<td>55%</td>
</tr>
</tbody>
</table>

Results: Basal evaluation was performed on 26 SR and 16 HR pts. Three SR pts with severe anemia (<7g/dl) and BMI=25 showed altered GLS at TP0. At the succeeding evaluation, 2 patients recovered, while one obese adolescent confirmed GLS and LVEF alterations. Follow-up after induction therapy (TP120) was performed on 10 SR pts and on 11 HR pts: GLS was altered in 3/10 SR pts (30%) and in 7/11 HR pts (63.6%). Two SR pts showed alterations in both GLS and LVEF values, while one child had isolated GLS impairment. Out of the 7 HR pts, one had both GLS and LVEF impairment and 3 worsened in GLS and LVEF values, while one child had isolated GLS impairment. Out of 42 patients, 32 (76%) underwent late-onset heart failure in both adult and pediatric ALL patients. Nonetheless, efficient strategies for the early diagnosis and management of cardiac toxicity during chemotherapy are still not defined.

Figure 1.

Summary/Conclusions: We established a cost-effective 10 color single tube FC-MRD assay with high sensitivity of least 1 in 105 and applicability in >97% BC-PALL MRD samples. We also described the frequency and extent of different cellular events and artifacts that can interfere with high-sensitivity BC-PALL 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 arrhythmogenic 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 arrhythmogenic toxicology, 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.

Methods: Rearranged products from within the TRG and TRB locus were generated by PCR using proprietary multiplex master mixes with consensus primers targeting all TRG and TRB V and J exons families, synthesized with MiSeq specific adapter 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.

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 comparing clonality with specific rearrangements was demonstrated between LymphoTrack® MiSeq and PCR-CE method.

Methods: Detection of clonality in clinical specimens from suspected B-cell malignancies using comprehensive IGH lymphotrack® MiSeq® and PGM® assays Y. Huang1, K. Hutt1, J. Panganiban1, A. Jacobsen1, N. Wong1, D. Duong1, R. Bob2, S.A. Pileri3, L. Bernard3, E. Gerbino3, T. Stenzel1, J.E. Miller1

1Invivoscribe Technologies, San Diego, United States, 2Department of Pediatrics, Korea University Guro Hospital, Korea University An聖on Medical Centre, Cha University, Seoul, Republic Of Korea

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).

Methods: Genotyping of NUDT15 was performed in 258 children with ALL who were registered in Samsung Medical Center. According to NUDT15 diplotypes, patients were classified into low risk (LR, wild-type), intermediate risk (IR, heterozygous variant), or high risk (HR, homozygous or compound heterozygous variant).

Results: A total of 182 were finally included after 76 patients were excluded for TPMT variation or lack of information during maintenance therapy; LR (n=131), IR (n=46), and HR (n=5).

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.
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 LYMPHOPROLIFERATIVE 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, CD20, CD38, CD58, CD13/33, CD66c, CD10, CD45 and CD34 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 precursor 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 IG/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 cytometry 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 unequivatable level. There was a strong correlation co-efficient between molecular and flow cytometric MRD analysis (R²=0.905, p<0.001). Correlation was strong with both IG/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 quantification of MRD and determination of MRD negative status. Flow cytometric methods may also permit MRD monitoring in patients where a suitable molecular assay cannot be developed.

E849

HYPOGLYCEMIC EVENTS DURING TREATMENT OF PEDIATRIC ACUTE LYMPHOPROLIFERATIVE 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 intensification phase (reinduction-consolidation) and during maintenance (MT). Pegylated asparaginase (PEG-ASP) was given in induction-consolidation phase of reintensification, as well as high-risk blocks. Additionally, the benefit of intensified 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=8), reinduction-consolidation (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 PNA regarding 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. Investigators' 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
1Pediatrics, Yeungnam University, College of Medicine, 2Pediatrics, Daegu Fatima Hospital, 3Pediatrics, Kyungpook National University College of Medicine and Dongsan Medical Center, 4Laboratory Medicine, Keimyung University School of Medicine, 5Laboratory Medicine, Yeungnam University College of Medicine, Daegu, Korea, Republic of Korea

Background: Acute lymphoblastic leukemia (ALL) is the most prevalent pediatric 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) is used for the backbone of ALL maintenance regimens. Genetic polymorphism in thiopurine methyltransferase (TPMP) is well known to affect the 6-MP tolerance. However prevalence of non-functional variant of TPMP 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. 6-MP dose intensity, defined as the ratio of prescribed 6-MP dose over protocol planned dose.

Results: We found two kinds of variants, c.55_G6insG/G6TGC(c869320776) in exon 1 from 8 patients and c.415C>T(rs116852322) in exon 3 from 14 patients. Of them, 7 patients had both variants and all variants were heterozygote. 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 smaller compared to other three genotypes (p=0.017). Frequency of febrile neutropenia, hepatotoxicity, cumulative days of antibiotics 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>Admissions during Maintenance</th>
<th>5-yr EFS (%)</th>
<th>5-yr OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>26</td>
<td>9 (35%)</td>
<td>13 (50%)</td>
<td>98.6 ± 2.5</td>
<td>98.2 ± 1.8</td>
</tr>
<tr>
<td>c.415C&gt;T</td>
<td>8</td>
<td>3 (38%)</td>
<td>78.3 ± 6.8</td>
<td>87.5 ± 11.7</td>
<td>100.0</td>
</tr>
<tr>
<td>c.55_G6insG/G6TGC(c869320776)</td>
<td>1 (12.5%)</td>
<td>18.0 ± 7.5</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: Genotyping of NUDT15 could be beneficial to predict the tolerable dose of 6-MP of pediatric ALL patients.

E851
Abstract withdrawn.

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 markedly improved for last centuries, but the improvement was mainly observed in children under 10 years old. In contrast, the treatment outcomes of ALL in adolescents and young adults (AYA) still lag behind 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 outcome 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-mercaptopurine, 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-asparaginase at least once in initial 2 months were considered to be treated as pediatric protocol, and those 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), 10-15 year old (76% vs 57%, P<0.0001), and 20-24 year old patients (51% vs 31%, P=0.0116). In univiable 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 widely used in the 2000s, and further study is needed to follow up the recent trend of treatment, and outcome as a result.

E853
AUTOLOGOUS TRANSPLANTATION 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 recent Russian 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-depended 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 assumption as most possible source of biases was tested on our simulation 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-myeloblastic BEAM conditioning was scheduled as intense intensification (+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 (TIIi) phenotype was verified in 56
(52.3%), mature (T-IV) - in 10 (0.9%), thymic (TII, CD1a+) ALL - in 41 pts (38.3%). T-lymphoblastic lymphoma (T-IVL ≤ 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-Bay test - PMB = 0.004, Cox model output: 1/HR = 15.9, P = 0.008. Simulation model for remission consists of 3 fractions: early (α = 10%, t = 0.05 m, δ = 0.2 m), normal (α = 57%, t = 0.28 m, δ = 1 m) and late remission (α = 33%, t = 1.31 m, δ = 2.2 m), for survival consists of 2 fractions: short life (α = 59%, t = 22 m), long life (α = 41%, t = 600 m). 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-Bay and Cox model show significant (PM = 0.50, PCM = 0.50, HR = 0.93) but LM plot demonstrates recognizable bias in transplanted patient group (Fig. 3). The second experiment supposed that the existed effect of aHSCT (HR = 0.5), N = 500, Mantel-Bay and Cox model would show significance, but hazard ratio was underestimated (PM = 0.03, PCM = 0.03, HR = 0.05). Most experiments were done for repeated simulation, which demonstrated a very good agreement of Mantel-Bay and Cox methods and their robustness.

Figure 1.

Summary/Conclusions: The effect of autologous HSCT in T-cell ALL was confirmed by usual analysis and by simulation experiments. It was shown that potential bias caused by no constant hazard rate cannot explain the magnitude of HSCT effect demonstrated on real data. LM plot could express small bias. Mantel-Bay and Cox model are robust against violation of constant hazard assumption and give very concordant outputs. Cox model underestimated the effect of time-depending factor in case of dropping hazard. Simulations model is a good instrument for testing tests in situations of deviation from theoretical assumptions.

E854

INDUCTION WITH TYROSINE KINASE INHIBITORS, CONSOLIDATION WITH FLUDARABINE, ARA-C AND DAUNOXOME FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANT 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. On the other hand, the concomitant combination of TKIs to conventional chemotherapy regimens greatly increases complete molecular responses, but at the price of significant toxicities and high rates of deaths due to toxicity.

Aims: We present here the preliminary results of a sequential therapeutic strategy starting with TKI (Dasatinib) as single agent induction until CR is achieved. Fludarabine (Flu), Cytarabine (Ara-C), Lymphosarcoma Daunorubine (DNX), FLAD regimen and Dasatinib were given as consolidation therapy, in order to maximize efficacy and reduce toxicity. Allogeneic stem cell transplantation (HSCT) was planned for all patients in MRD negative CR.

Methods: Dasatinib was given in association with steroids at the dosage of 140mg id/le until the achievement of CR. FLAD regimen consisted of a three days administration of Flu 30mg/sqm followed by Ara-C 2000mg/sqm and DNX 100mg/sqm. 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/ABL.

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/ABL was still positive both on day 33 and on day 70. Therefore, we found 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/ABL 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 4 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 course in molecular CR because of an unrelated event.

Summary/Conclusions: This therapeutic strategy proved to be well tolerated and extremely effective for Ph+ ALL patients. Administering FLAD in patients who had already achieved complete hematological response with Dasatinib + steroids allowed us to reduce the period of neutropenia and thrombocytopenia compared to what is reported after combined TKI and chemotherapy treatment given at diagnosis. Most patients underwent HSCT in molecular CR.

E855

BONE MARROW MRD EVALUATION ON DAY 7 OF STEROID TREATMENT OF MODIFIED ST JUDE TOTAL XV THERAPY IN STANDARD/LOW RISK PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: In the recent years it was clearley shown that levels of minimal residual disease (MRD) studied by flowcytometry during treatment reflect the overall response to the chemotherapy and give a chance to individualize treatment and improved outcome.

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 fulls with high dose methylprednisolone (HDMXP) we add 7 days of HDMXP to the modified St Jude Total XV as an initial treatment and randomized patients at doses of 10mg/kg/d or 20mg/kg/d HDMXP: not exceeding at maximum 1000mg methylprednisolone. 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 analyse 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 6400/mm3 (110-55300/mm^3),all were Calla+ pre B cell ALL (17 low risk ALL, 6 standart 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 HDMXP and 12 patients were in the group of 20mg/kg/d HDMXP. 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/mm^3. 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 all were steroid responsive.

Summary/Conclusions: Our preliminary results suggest to think that MRD level on day 7 in a small group of low/standart ALL patients may not predict outcome.
and clinical outcomes of the patients were analyzed. Lymphoblastic leukemia (ALL, n=46) were enrolled in this study. Expression of JL1 antigen is expressed on tumor cells of T, B, and myeloid lineage in >80% of acute leukemia patients, and its expression is limited in normal multipotent hematopoietic cells. The antigen is not expressed on mature peripheral blood lymphocytes (LyBP) in over 99% of healthy people. JL1 is a novel epitope of CD43, which is known to be specifically expressed on lymphoblastic leukemia (LLyBP). The antigen is expressed on tumor cells in >80% of pediatric acute lymphoblastic leukemia (ALL) patients and to assess their correlation with baseline characteristics and prognostic factors.

Aims: The aim of this study was to evaluate baseline levels of cytokines, cytotoxic 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 in this study. We evaluated serum levels of 31 analytes were correlated with baseline characteristics and prognostic factors, such as the number of baseline analyte levels and inferior PFS or OS were found. In newly diagnosed ALL patients, we found statistically significant correlations between sTN-Fr-1 and sTNFR-2 (r=0.805; p<0.0001), IL-1α and IL-4 (r=0.700; p=0.008), sTNF-β and VEGF (r=0.664; p=0.012). Furthermore, we found significant positive correlations between IL-7 and antithrombin III (r=0.758; p=0.075). These findings demonstrated that anti-JL1 antibody might be used in childhood acute leukemia patient showing JL1 expression.

Results: Positive JL1 expression was observed in 16 (64.0%) patients with AML, and 27 (58.7%) with ALL. In AML patients, positive JL1 group showed higher expression than negative JL1 group in CD 14 (P=0.043), CD7 (P=0.026), CD56 (P=0.016) and lower expression in CD65 (P=0.05). With regard to ALL patients, CD 20 (P=0.002) and CD22 (P=0.005) expressions were significantly higher in JL1 positive group than JL1 negative group. Positivity of JL1 expression did not show significant difference between B-lineage vs. T-lineage ALL (P=0.671). Positivity of JL1 expression was not significantly associated with overall survival in 71 patients with newly diagnosed childhood acute leukemia (P=0.570).

Summary/Conclusions: 60.5% of childhood acute leukemia displayed positive JL1 expression. This finding is similar to 61.2% of JL1 expression in adult AML and 57.9% of expression in adult ALL reported previously. The JL1 expression was significantly associated with some immunophenotypic features, but was not significantly associated with clinical outcome. These findings demonstrates that anti-JL1 antibody might be used in childhood acute leukemia patient showing JL1 expression.

E857

JL1 ANTIGEN EXPRESSION OF LEUKEMIC CELLS IN CHILDHOOD ACUTE LEUKEMIA

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Background: JL1 is a novel epitope of CD43, which is known to be specifically expressed depending on the differentiation stages of hematopoietic cells. JL1 antigen is expressed on tumor cells of T, B, and myeloid lineage in >80% of acute leukemia patients, and its expression is limited in normal multipotent hematopoietic cells. The antigen is not expressed on mature peripheral blood cells or other normal tissues. Thus, the clinical phase 1 test of a therapeutic agent for leukemia targeting JL1 is being conducted, and when anti-JL1 antibody was combined with a toxic substance, its therapeutic effect was found earlier in preclinical trials.

Aims: This study aims to examine JL1 expression of leukemic cells in childhood acute leukemia.

Methods: Between December 2014 and January 2016, a total of 71 patients younger than 21 years with acute myeloid leukemia (AML, n=25), and acute lymphoblastic leukemia (ALL, n=46) were enrolled in this study. Expression of JL1 was assessed using a commercially available kit (Bio-Rad, CA USA) based flow cytometry, and an expression of 20% or above was defined as positive JL1 expression. Pathological and immunophenotypic characteristics, and clinical outcomes of the patients were analyzed.

Results: Median duration of PON therapy was 3 months (5 days-30 months+). Out of the 19 pts who received PON for ≥ 4 weeks, 5 pts failed to reach CR, 14 (73.7%) 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 trombo-embolic event; no arterial occlusive event). Post-induction therapy consisted in PON-based therapy in most pts. HSCT was performed in 5 pts. Out of 13 pts in CR on PON, 1 pt died in CR from acute lymphoblastic leukaemia relapse 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, respectively.

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-agent for leukemia targeting JL1 is being conducted, and when anti-JL1 anti-
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 Hokuyu Hospital. The patients’ eligibility were 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 early course were treated without TKIs (non-TKI group). Overall survival was 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 did not differ between the two groups when we performed univariate analysis. However, by multivariate analysis using Cox regression model for adjusting confounding factors, including, overall survival was superior for IMA-pts [Hazard 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) 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 hematopoietic 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 pts were treated according to RALL–2009 protocol (ClinicalTrials.gov; NCT00333993) 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.

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 autologous 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 35 pts constituted 54.6% and 40.4% respectively. The long-term outcome on both protocols 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 land mark 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 non-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 (45y) 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 positive. 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 blinatamobabel 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-positive 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. Palmar-plantar eruption was noted in one pt on sorafenib completely resolved after temporarily TKI discontinuation. Diarrhea 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-regulatory lymphocytes 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 exhaustion. T-cytotoxic and NK cells subpopulations restoring also correlated with clinical effectiveness.

Figure 1.

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 positive. 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 blinatamobabel 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-positive 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. Palmar-plantar eruption was noted in one pt on sorafenib completely resolved after temporarily TKI discontinuation. Diarrhea 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-regulatory lymphocytes 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 exhaustion. 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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Results: During this period, a total number of 353 patients with childhood ALL were treated in our Department, according to BFM protocols. Recurrence occurred in 86 patients (24.4%, 56 male - 30 female - median age: 4.83 years), within 3 to 184 months from initial diagnosis. Very very late recurrence was noted in 3.1% of our relapses (8 male - 3 female) at 53, 72, 83, 84, 108, 112, 116, 120 and 184 months from initial diagnosis. In 9 patients recurrence involved the bone marrow, in 1 both bone marrow and central nervous system (CNS) and in 1 only the testicles. Two children had received allogeneic BMT from a matched related donor in first complete remission (CR1) and they had a bone marrow relapse 4 and 5 years later, respectively. The mean WBC, Hb, Blasts and PLT values at diagnosis were 29260/mm3, 5.6g/dl, 21360/mm3 and 18000/mm3, respectively. All of them were B-cell ALL except for 1 who had CD33 and CD13 co-expression. Regarding the immunophenotypical profile of the disease at recurrence, it remained almost identical to the initial. Regarding cytogenetic characteristics of the patients at diagnosis, 3 of them had high hyperdiploidy, one del(6)(q12), one BCR-ABL fusion and one 47,X,Y+13(9), idio- del(12p)(q13?); none del(12p). In ALL, the disease at recurrence, the cytogenetic profile remained identical at recurrence, while in 1, trisomia 13 was not detected and another had heterozygous absence of IKZF1, PAIX5, EBF1, CDKN2A and CDKN2B genes. On Day 8, nine of 11 patients were Prednisone Good Responders. On Day 15, nine children had bone marrow m1, one m2 and one m3, and on Day 33 only one had m2. Two patients were classified as low risk, 6 as intermediate risk and 3 as high risk. Second remission (CR2) was achieved in 9 children with very very late recurrence. The other 2 died from disease progression. Six of nine patients are still alive and well 6, 8, 10, 10, 11 and 20 years after initial diagnosis. One patient of 9 died from secondary recurrence and the last two had a second allogeneic BMT and died due to severe infection, 2 and 11 months following BMT. Interestingly, 3 out of 5 patients who finally died, had the very very late recurrence (10, 10 and 15 years after initial diagnosis) and had been treated with adult type protocols.

Summary/Conclusions: The rate of very very late B-cell ALL recurrence was only 12.8% of all recurrences. The prognosis is worse in patients, older than 18 years, treated with adult type protocols.
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.

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: Recently, comprehensive genetic profiling of pediatric and adult core-binding factor (CBF) AML revealed a variety of cooperating events in a cohort of 85 t(8;21) AML patients (Faber et al. Nat Genet 2016). These mutations comprised alterations in genes encoding for proteins in tyrosine kinase (TK) signaling, epigenetic regulation (ER), and in the cohesin complex (CC).

Aims: To validate and to further extend our recent findings by comprehensive characterization of the mutational landscape of t(8;21) positive AML using a high-throughput targeted sequencing (HTS) approach.

Methods: The HTS panel comprised the entire coding region of 244 genes that are involved in hematological malignancies. Pretreatment blood (n=23) or bone marrow specimens (n=72) of 95 additional adult t(8;21) positive AML patients (pts) (median age: 51 yrs, range 18-72 yrs) were analyzed. 92/95 pts were enrolled in one of seven prospective AMLSG treatment trials. Libraries (total probe size: 1.359 Mbp) were prepared using SureSelectXT custom solutions (Agilent). Paired-end sequencing was carried out on a HiSeq 2000 (Illumina). The variant allele frequency (VAF) cutoff for reporting mutations was set at ≥0.05.

Results: The median coverage per pt was 900x. Mutations were detected with an average of 5.1 (SD: ±2.6) per pt with 99% of all pts harboring at least 1 mutation and 87% ≥ 3 mutations. Consistent with previous studies, mutations in TK signaling pathways were common events: KIT mutations were found in 22/95 pts (23%) followed by mutations affecting NRAS (16/95; 17%), FLT3 (11/95; 12%; point mutations only), and KRAS (4/95; 4%). A significant enrichment of mutations was also observed in genes involved in epigenetic regulation, ASXL1 (15/95; 16%), ASXL2 (12/95; 13%), KDM6A (11/95; 12%), CREBBP (8/95; 8%), SRCAP (8/95; 8%), EZH2 (7/95; 7%), SETD2 (5/95; 5%), TET2 (12/95; 13%) and DNMT3A (5/95; 5%), highlighting their contribution in altering the epigenetic state of this leukemia subtype. Moreover, mutations affecting members of the CC were found with a high frequency: RAD21 (13/95; 14%), SMC1A (5/95; 5%), STAG2 (3/95; 3%), and SMC3 (2/95; 2%). Of note, mutations in CC genes were almost mutually exclusive. We also identified additional mutations in previously detected cooperating genes such as mutations clustering in exon 2 of the ZBTB7A gene (15/95; 16%), encoding for a transcription factor involved in hematopoietic lineage fate. Recurrent mutations were also observed in CCL22 (9/95; 9%), that plays an important role in regulation of hematopoietic cell proliferation, as well as DDX15 (6/95; 6%) being involved in spliceosome function and ribosome biogenesis. With respect to the clonal architecture we found that the median VAF in genes belonging to ER and CC (0.30; range 0.03-0.91; 0.31, range 0.05-0.73, respectively) was higher than in genes associated with TK signaling (0.19, range 0.05-0.53). These data suggest that alterations affecting the epigenetic state and differentiation occur earlier than those in signaling during t(8;21) leukemogenesis.

Summary/Conclusions: Using a comprehensive, deep sequencing approach we could further characterize the mutational landscape of t(8;21) positive AML. Here, mutation clusters in genes involved in TK signaling, ER and CC were confirmed as well as novel CBF-associated gene mutations that play an essential role in regulation of hematopoietic cell proliferation and differentiation. Further analyses in terms of sample size extension as well as correlation of findings with clinical parameters are ongoing.
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. We 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 leukemia 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 (IBI): 0.25, 0.5, 1.0 and 2μM) in combination with Daunorubicin (DAU 5nM) or RAC (NSC 23766 (NSC): 5, 10, and 20μM) for 48 hrs and cell viability was assessed using Annexin V/Sytox-Blue based flow cytometric analysis.

Results: In the context of 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 leukemia 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, indicated down-regulated p65 upstream of BTK in the context of MA9.3 treatment. 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 in 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 that RAC-GTPase signaling may also represent a target in AML, particularly in the context of FLT3-ITD (Longo 2009 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 IBR/DAU significantly reduced RAC activation, positioning BTK upstream of RAC in line with observations reported earlier. We further 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. Abruptly 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.

E867
SECRETION OF SOLUBLE FACTORS BY AML CELLS INFLUENCE CD3/CD3 BITE® ANTIBODY MEDIATED CYTOTOXICITY AND T-CELL PROLIFERATION

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Background: In our previous work, we showed that the CD3/CD3 BITE® antibody construct (AMG 330) is able to recruit autologous, residual T cells and induce cytotoxicity against primary AML cells ex vivo. However, as described previously (Musasi et al, Blood 2013) primary AML cells are able to secrete soluble factors, which might not only influence T-cell proliferation but also negatively affect AMG 330 mediated cytotoxicity.

Aims: In this study we characterized the influence of soluble factors secreted by primary AML cells on AMG 330 mediated cytotoxicity.

Methods: We used plasma samples (from heparinized serum tubes or after density gradient centrifugation) from newly diagnosed and relapsed AML patients in AMG 330 cocultures of healthy donor (HD) T cells and AML cell lines. In flow cytometry based experiments we determined the influence of AML plasma in comparison to fetal calf serum (FCS, heat inactivated) on AMG 330 mediated cytotoxicity and T-cell proliferation. In transwell experiments using primary AML cells physically separated from AMG 330 cocultures, we evaluated if AML cells are the source of soluble factor secretion.

Results: The influence of AML plasma from bone marrow (BM) of AML patients on AMG 330 mediated cytotoxicity and T-cell proliferation was heterogeneous: In 15/30 samples, AMG 330 mediated cytotoxicity was significantly reduced compared to cultures containing FCS (mean% specific lysis FCS vs BM: 72.8 ± 26.8). There was an associated reduction in T-cell proliferation (mean% proliferation FCS vs BM: 27.7 ± 9.5%). The degree of immunosuppression could not be correlated to percentage of bone marrow blasts. Interestingly, the effect was not observed in AML plasma samples from peripheral blood (PB) (mean% specific lysis FCS vs PB: 84.7 ± 83.5%, proliferation FCS vs PB 32.2 ± 15.9%). In the remaining 15 samples, plasma from AML BM influenced AMG 330 mediated T-cell function was observed (mean% specific lysis FCS vs BM: 82.1 ± 78.3%; proliferation FCS vs BM: 25.7 ± 26.8). In control cultures plasma from AML patients in complete remission (CR) or from HD BM was used which did not negatively impact AMG 330 mediated cytotoxicity (mean% specific lysis FCS vs CR: 76.5 ± 69.2%; FCS vs PB: 76.1 ± 76.2%), proliferation (FCS vs CR: 76.5 ± 68.2%; FCS vs HD: 58.9 ± 65.4%). To further explore the influence of soluble factors from primary AML cells, we performed transwell experiments. Primary AML cells were cultured in the previously described long term culture system (Krupka et al., Leukemia 2016) and HD T cells were added. In all MOLM 13 cells were placed in transwell devices (3μm). In analogy to our findings with AML BM plasma, we observed a strong reduction in AMG 330 mediated cytotoxicity and T-cell proliferation in 7/14 experiments (mean% specific lysis control vs AML: 95.0 vs 70.8%; proliferation control vs AML: 78.6 vs 26.8).
E868

CLONAL EVOLUTION OF FLT3-ITD POSITIVE AML AT DIAGNOSIS AND RELAPSE IN PATIENTS TREATED WITHIN THE CALGB 10603 (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. Treatment options for FLT3-ITD carry a high risk of resistance and the role of a multi-targeted TKI (midostaurin; 6 pts in the AMLSG16-10 (NCT01477606) 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 (illumina) for library preparation followed by sequencing on an Illumina HiSeq2000. 6 pts were treated in the RATIFY trial receiving either midostaurin or placebo combined with intensive chemotherapy during induction and 11 pts were treated in the AMLSG16-10 trial. In the former treatment with midostaurin 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, hematopoietic 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 the original ITD and 1 pt gained an additional ITD clone at Rel. 3 pts had a D835V FLT3-TKDmut 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 and Rel). 40 mut were found only at Dx, 73 (24%) mut were detected only at Rel and 14 mut with 10 mut only 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 mTOR switch under stromal influence. These observations suggest that resistance to targeted therapies in vivo in part may derive from changes that AML cells induce in the microenvironment.

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 in culture. Although some of the components of the stromal secretome (the totally 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 cell survival; (ii) investigate global changes in signaling pathways activity induced by stromal factors in primary AML; (iii) Validate the functional significance of these interactions through targeted inhibition of BMSM activated signaling pathways.

Methods: We used primary AML cells and established cell lines. Four different human AML lines 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 secretome (in triplicate). Proteins in these secretomes were quantified using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Peptide sequence searches against both mouse and human proteomes allowed for discrimination between the mouse stromal and human AML proteins. Guava EasyCye Flow Cytometry was used to measure the viability and proliferation of these cell populations, assessing the capability of the identified factors above on primary AML cells (n=6) as well as the effects of kinase inhibitors (midostaurin, trametinib, tofacitinib and Midostaurin over 72hrs with and without stromal conditioned media (n=10). Label-free LC-MS/MS based phosphoproteomics quantified >5,000 phosphorylation sites in primary AML patient cells treated with identified stromal factors individually and combined in triplicate. Commercial (MASCOT) and in-house (PESCAL, KSEA) software were utilized to identify and quantify proteins, determine kinase activities and interpret intracellular signaling.

Results: Initially by comparing secretomes 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 collagen, a secreted extracellular matrix protein [CTGF] and bone morphogenic protein-1 [BMP-1]) based on their ability to effect growth and likely signalling capacity in 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 secretome 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 could induce sensitivity to another kinase inhibitor and insensitivity towards the previously effective inhibitor.

Summary/Conclusions: This proteomic approach has allowed identification of a panel of key proteins (including 10-A11, CTGF, BMP-1) secreted by the stromal cells that modulate cell signalling and cell fate in AML blasts. Using a multi-targeted approach to screen secreted stromal factors we were able to dissect the factor specific effects on AML signalling. Subsequent survival assays and targeted inhibition studies demonstrate that despite heterogeneity in patient response to these factors, activity in key signalling pathways such as mTOR and mTOR switch under stromal influence. These observations suggest that resistance to targeted therapies in vivo in part may derive from changes that AML cells induce in the microenvironment.

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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Methods: Initially by comparing secretomes 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 collagen, a secreted extracellular matrix protein [CTGF] and bone morphogenic protein-1 [BMP-1]) based on their ability to effect growth and likely signalling capacity in 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 secretome 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 could induce sensitivity to another kinase inhibitor and insensitivity towards the previously effective inhibitor.

Summary/Conclusions: This proteomic approach has allowed identification of a panel of key proteins (including 10-A11, CTGF, BMP-1) secreted by the stromal cells that modulate cell signalling and cell fate in AML blasts. Using a multi-targeted approach to screen secreted stromal factors we were able to dissect the factor specific effects on AML signalling. Subsequent survival assays and targeted inhibition studies demonstrate that despite heterogeneity in patient response to these factors, activity in key signalling pathways such as mTOR and mTOR switch under stromal influence. These observations suggest that resistance to targeted therapies in vivo in part may derive from changes that AML cells induce in the microenvironment.
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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. FLT3is 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 trial and AMLSG 16-10 (NCT01477606) trial with regard to AR-FLT3-ITD and FLT3-ITDmut, 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 entering the RATIFY trial either had either a FLT3-ITDmut (n=22), as FLT3-ITDmut (n=2), or both (n=2). The median AR of FLT3-TKDmut at Dx was 0.82 (0.27-2.66) 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 (36%) pts. There was no significant difference between the median FLT3-ITD AR at Dx [0.62 (0.10-18.94)] and Rel [0.65 (0.07-38.75); p=0.98]. A switch of the ITD clone 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 [0.62 (0.05-8.91); 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 Rel, indicating the preeminence of ITD clones in the context of resistance to 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.

E872

COOPERATION OF MLL-PTD WITH DNMT3A OR RUNX1 MUTATIONS IN AML LEUKEMOGENESIS

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Background: Our previous study showed that DNMT3A or RUNX1 mutations were frequently coexisted in the MLL-PTD AML patients (Oncoarget 2015). We aimed to investigate the role of coexisted DNMT3A or RUNX1 mutations in leukemogenesis of MLL-PTD AML.

Methods: After lentiviral-mediated over-expression of RUNX1 or DNMT3A mutants in MLL-PTD mouse bone marrow (BM) cells or human MLL-PTD AML cells, colony formation, cell proliferation, differentiation and apoptosis assays were carried out. Interaction of RUNX1, HIF-1α, and MLL-PTD were evaluated by co-immunoprecipitation assay. Differential gene sets and protein expression, histone modifying protein expression, and enrichment of histone 4 acetylation (H4Ac) were assessed by RT-qPCR, Western blot, and ChIP-qPCR, respectively. For BM transplantation assays in mice, MLL-PTD BM cells over-expressing DNMT3A-Wild type (WT) mutants and empty vector (EV) control were injected into C57BL/6 mice via tail vein.

Results: We observed that MLL-PTD BM cells with RUNX1 mutants lacking C-terminal WWRPY sequence (H377SxK and V435fsX376) had increased self-renewal, proliferation, increased HIF-1α and its downstream gene expression. In addition, the interaction of HIF-1α and MLL-PTD was disrupted in the cells transduced with C-terminal truncated RUNX1 mutants or RUNX1 shRNA. Compared with those expressing DNMT3A mutants, over-expression of DNMT3A-Wt reduced cell growth, colony formation and self-renewal activities of EOL-1 and MLL-PTD BM cells. All DNMT3A mutants impaired Nαbulyrate-induced differentiation, but only R82H mutant impaired ATRA-induced differentiation in vitro. DNMT3A mutations were associated mostly with up-regulation of homeobox B (HOXB) genes. The expressions of BCL2, AREG, PRKCA, PRKN were all up-regulated and MCL1 was significantly down-regulated in the DNMT3A-mutant cells compared to those of DNMT3A-Wt. Cells with DNMT3A- mutants showed regulation of H4Ac enrichment at the HOXB and HOXC12 promoter regions compared to the control cells or the cells with DNMT3A-Wt. DNA methylation microarray analysis identified both hypo- and hypermethylation features in different regions throughout the genome of DNMT3A-mutant-transduced EOL-1 cells. Up-regulated genes including HLX and HBEF1 were hypermethylated in the EOL-1 cells transduced with DNMT3A mutants. In vivo study showed that white blood cells including neutrophils, lymphocytes and monocytes increased significantly (P<0.03) in the DNMT3A-mutants mice compared to EV or WT-mice at 10 months post-BM transplantation.

Summary/Conclusions: The present study showed that both RUNX1 and DNMT3A mutants dysregulated self-renewal, proliferation and apoptosis in the mouse MLL-PTD BM cells. Disruption of MLL-PTD-RUNX1-HIF-1α complex in the RUNX1-mutant and aberrant methylation in the DNMT3A-mutant cells might play an important role in AML pathogenesis. Our results showed that cooperatively RUNX1 or DNMT3A mutations had impact on leukemogenesis of MLL-PTD AML.

E873

AML BLASTS INDUCE A SENESCENT PHENOTYPE IN THE BM-MSC

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Background: Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder that arises from the haematopoietic myeloid progenitor cells within the
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 therein. The microenvironment promotes 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-tumoural 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 (LREC/07/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 Isolation Kit (Miltenyi Biotech). AML blasts cultured in co-culture with 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 assayed by β-Galactosidase staining.

**Results:** From the RNA sequencing data 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 (2.636 logFC). p21 mRNA expression was confirmed by RT-PCR in AML BM-MSC compared to normal BM-MSC. In-vitro experimentation showed that p21 mRNA and protein expression is increased in BM-MSC when co-cultured with primary AML. Furthermore, we show that AML increased senescence β-Galactosidase staining in BM-MSC and that p21 knockdown in BM-MSC reversed the senescence 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. In identifying this novel microenvironment feedback loop in AML we highlight a potential new target for future AML therapies.
a preferential engraftment in the presence of FLT3-ITD mutation (9 of 18). Furthermore, we found that the mutational fraction 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 xenotransplants. 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% in healthy pre-treated ALL patients, reasons for resistance have not been determined. In contrast to classical T-cell activation, BiTE® antibody construct mediated T-cell activation relies solely on binding to the CD3ε chain of the T-cell receptor (TCR) complex. Resolving the exact mechanism of BiTE® antibody construct mediated T-cell activation is a prerequisite for our understanding of mechanisms of resistance. Aims: In the present study we characterized the role of costimulation on intracellular signalling in CD33/CD7 BiTE® antibody construct (AMG 330)-mediated T-cell activation.

Methods: We generated a murine cell line stably expressing human CD33 and devoid of human costimulatory molecules (B33). In in vitro cocultures, cytokicity against B33 cells and the AML cell line MOLM-13 was evaluated by flow cytometry, Activation of downstream signalling pathways was assessed by a phospho-flow cytometry protocol for T-cell recruiting antibodies. Results: Coculture of B33 cells with CD3+ healthy donor T cells (n=4) resulted in AMG 330 mediated mean cytokicity of 58.3%. In contrast, MOLM-13 cells were completely lysed (% specific lysis relative to control B33 vs MOLM-13: 58.3±32.9 vs 99.9±0.1, n=4), despite comparable CD33 expression levels (CD33 medium fluorescence intensity (MFI) ratio: B33 116.1 vs MOLM-13 67.6). However, with the addition of an antiCD3/antiCD28 antibody construct, mean% cytokicity against B33 cells could be restored (% specific lysis AMG 330 vs anti-CD3/anti-CD28: 1.4±1.2 and 3.0±1.0 respectively, n=3). At lower E:T ratios (1:4) the additional costimulatory signal also increased AMG 330 mediated mean% pAkt and pErk (mean% phosphorylated (p)Akt and pErk 7.9 and 7.6, vs 1.7±1.9 and 3.5±2.0, respectively, n=3) (% specific lysis AMG 330 vs anti-CD3/anti-CD28: 65.1±19.7 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 MOLM-13 cells in the absence of antiCD3/antiCD28 antibodies semi-de novo. 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=3-5) 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).

Summary/Conclusions: Our data support the hypothesis that costimulation influences the susceptibility of target cells to lysis by T-cell recruiting antibody constructs. Currently, we are validating our results in a larger cohort using T cells from healthy donors and patients with AML. Furthermore, we will analyse the phosphorylation pattern within different T cell subsets and upon knock out of different CD3+ subsets. Our results will contribute to the understanding of BiTE® mediated activation of T cells, which is a prerequisite for clinical responses.

E878

ESTABLISHING SINGLE CELL WHOLE EXOME SEQUENCING ANALYSIS AS A DISCOVERY TOOL IN NPM1/FLT3-POSITIVE PEDIATRIC ACUTE MYELOID LEUKAEMIA

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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 whole exome sequencing (WES) of single 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 amplification 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 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 three sequencing approaches. WES readily identified n=103 (85.1%) and n=93 (78.9%) of all 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 cells invade non-hematopoietic tissues and form solid tumors. It may occur as isolated event or simultaneously with leukemic 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.

Aims: 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 and BM carried out using next generation sequencing approach. WES of amplified single cell DNA was 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 three sequencing approaches. WES readily identified n=103 (85.1%) and n=93 (78.9%) of all 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.

Results: This study comprised 14 patients with MS (MS-group) and 14 patients with AML without any evidence of extramedullary involvement (BM-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 (mean of MS group vs non MS group, 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 in vitro 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 suggest 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 harboring Nucleophosmin1 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 LC3II/LC3I 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 autophagy 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 potential therapeutic target in AML.

E881

BY AN MCL-1-DEPENDENT MECHANISM, ALVOCIDIB POTENTIATES THE ACTIVITY OF CYTARABINE AND MIOTOXANTRONE WHEN ADMINISTERED IN A TIME SEQUENTIAL REGIMEN IN AML

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Background: Treatment with alvocidib has shown significant improvements in the complete remission rates in newly diagnosed acute myeloid leukaemia (AML) patients when administered before cytarabine and mitoxantrone (ACM regimen) in a randomized Phase 2 study compared to 7+3. Although the mechanism of alvocidib action as a single agent is documented, the mechanism underlying synergy found in the ACM regimen is not fully understood. The ACM regimen was originally developed based on the perceived benefit of a time-sequence regimen starting with cell-cycle arrest (alvocidib), followed by release of the cells from arrest and inhibition of DNA replication (cytarabine/mitoxantrone) during S-phase. However, recent reports suggest that the transcriptional repression of key anti-apoptotic proteins (eg., MCL-1) mediated by alvocidib’s CKD9 inhibition, may contribute to the activity in the ACM regimen.

Aims: We hypothesized that MCL-1 transcriptional repression constitutes the primary mechanism for the synergism observed with the ACM treatment regimen.

Methods: Following treatment, cell viability and caspase activation, an indicator of apoptosis, were assessed using CellTiter-Glo and Caspase-Glo assays, according to manufacturer protocol. mRNA levels were assessed using RT-PCR. Protein levels were assessed using standard immunoblotting technique.

Results: In this study, we demonstrate that treatment with alvocidib, followed by treatment with cytarabine and mitoxantrone, synergized in vitro and correlated with the downregulation of MCL-1 protein and mRNA expression. Indeed, the ACM regimen resulted in a 2.4 or 3.4-fold increase in caspase activity relative to any single agent within the combination in MV4-11 or OCI-AML3 cells, respectively. As has been previously reported, we also observed that increased activity of cytarabine in alvocidib-treated cells corresponded with progression into the S-phase of the cell cycle, following the washout of alvocidib. However, this observation accounted for only a small portion of the inhibition of cell proliferation. This was further confirmed by the observation that CDK4/6 (cell cycle) specific inhibitors, such as palbociclib, did not show synergistic increases in caspase activity following treatment in the same setting. In various AML cell lines treated with MCL-1 siRNA, followed by cytarabine and mitoxantrone treatment, we also observed a synergistic increase in the inhibition of cell proliferation.

Summary/Conclusions: Considering our earlier work showing that MCL-1 dependence predicts AML patient response to the ACM regimen, we propose that MCL-1 repression is the primary mechanism of alvocidib’s clinical activity. As MCL-1 also confers resistance to cytarabine, the current study provides additional rationale for the inclusion of alvocidib in the treatment of AML, and in the ACM regimen specifically. Taken together, this data suggests that the ACM regimen may be an effective regimen in treating patients with high-risk AML because of alvocidib’s inhibition MCL-1.

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
patients, we perform Microarray-based High-Throughput Technology with Affymetrix SNP array 6.0 or Cytoscan HD. Survival data were collected prospectively from the time of diagnosis, with a median follow-up of 18 months. Survival analysis was performed with Kaplan Meyer method using log rank test. Univariate and multivariate regression and Cox Hazard Ratio (HR) model was performed. Correlation between variables was assessed with Fisher’s exact test. Results: Autophagy alteration (gene group 1: M238; gene group 2: M16) had 14 and 2 pairs with significant expression, respectively. CEBPA (gene 911: U1K7R17; BECN1; ATG14; AMBRA1; UVRAG; ATG9A; ATG9B; PIK3C3; PIK3R4) were related to lower Complete Remission rate (CR%) after induction in univariate (p<0.001) and multivariable regression with age, karyotype, secondary AML, TP53 mutation (p<0.01). Autophagy alteration showed to confer worse OS (p<0.001) and was significantly associated with complex karyotype and TP53 mutation (p<0.001). We detected significant differences in terms of survival independently both in Copy Number (CN) Gain and CN Loss in group 1 genes (p<0.001). Furthermore, we investigated genes in AMPK pathway (2: S6EN1; PRKAA1 CHR 3; PRKAB1; PRKAA1 CHR 1; PRKAG1 CHR11; PRKAG1 CHR11): and other genes that could contribute to a switch from a physiological role of autophagy to a resiliency mechanism (group 3: CCND1; BCL2; PINK1; PARK2; TP53; MDM1; MDM4): alterations in those genes were shown to confer worse OS (p<0.001 in both groups). Alteration in group 2 and group 3 were related to lower CR% after induction (p<0.001 in both groups). Whole Exome Sequencing on 56 patients in our set did not found any significant mutation in genes we analyzed with the exception of TP53.

Summary/Conclusions: Our work investigates for the first time with a genomic approach the role of autophagy in AML. We found that both CN gain and CN loss in autophagy key regulator genes are associated with poor prognosis and therapy resistance. A CN loss in autophagy could enhance proliferation and block apoptosis, a CN gain could give cell resiliency, favoring cytokopten turnover, damaged mitochondria elimination, and neutralizing oxidative damages. Further functional studies will be necessary in order to confirm these results.

Acknowledgements: ELN. ALL, AIRC, PRIN, Progetto Regione-Università 2010-12, FP7 NGS-PTL project. HARMONY.

E883
NO EVIDENCE FOR MICROSATELLITE INSTABILITY (MSI) IN 1,394 PATIENTS (PTS) WITH ACUTE MYELOID LEUKEMIA (AML)
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Background: MSI is the addition or loss of bases within repetitive DNA sequences located in microsatellites (MS) found in DNA repair. MSI is most often observed in endometrial and colorectal carcinomas, and pts with MSI-positive (MSi) solid tumors have shown promising responses to therapy with immune checkpoint inhibitors. MSI’s existence in AML has been examined in several studies, but the results are equivocal, with some reporting a total absence of MSI and others finding as many as 20% of AML pts to be MSI+. These studies tested for MSI using polymerase chain reaction (PCR) analysis was performed with Kaplan Meyer method using log rank test. Univariable and multivariable regression and Cox Hazard Ratio (HR) model was performed. Correlation between variables was assessed with Fisher’s exact test. Results: Autophagy alteration (gene group 1: M238; gene group 2: M16) had 14 and 2 pairs with significant expression, respectively. CEBPA (gene 911: U1K7R17; BECN1; ATG14; AMBRA1; UVRAG; ATG9A; ATG9B; PIK3C3; PIK3R4) were related to lower Complete Remission rate (CR%) after induction in univariate (p<0.001) and multivariable regression with age, karyotype, secondary AML, TP53 mutation (p<0.01). Autophagy alteration showed to confer worse OS (p<0.001) and was significantly associated with complex karyotype and TP53 mutation (p<0.001). We detected significant differences in terms of survival independently both in Copy Number (CN) Gain and CN Loss in group 1 genes (p<0.001). Furthermore, we investigated genes in AMPK pathway (2: S6EN1; PRKAA1 CHR 3; PRKAB1; PRKAA1 CHR 1; PRKAG1 CHR11; PRKAG1 CHR11): and other genes that could contribute to a switch from a physiological role of autophagy to a resiliency mechanism (group 3: CCND1; BCL2; PINK1; PARK2; TP53; MDM1; MDM4): alterations in those genes were shown to confer worse OS (p<0.001 in both groups). Alteration in group 2 and group 3 were related to lower CR% after induction (p<0.001 in both groups). Whole Exome Sequencing on 56 patients in our set did not found any significant mutation in genes we analyzed with the exception of TP53.

Summary/Conclusions: Our work investigates for the first time with a genomic approach the role of autophagy in AML. We found that both CN gain and CN loss in autophagy key regulator genes are associated with poor prognosis and therapy resistance. A CN loss in autophagy could enhance proliferation and block apoptosis, a CN gain could give cell resiliency, favoring cytokopten turnover, damaged mitochondria elimination, and neutralizing oxidative damages. Further functional studies will be necessary in order to confirm these results.

Acknowledgements: ELN. ALL, AIRC, PRIN, Progetto Regione-Università 2010-12, FP7 NGS-PTL project. HARMONY.

E884
SY-1425, A POTENT AND SELECTIVE RARA AGONIST, REPROGRAMS AML CELLS FOR DIFFERENTIATION ALONG DISTINCT LINEAGES, UNCOVERING PD MARKERS FOR CLINICAL STUDIES
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Background: SY-1425 (famibartotene) is a potent and selective agonist of the retinoic acid receptor alpha (RARA) transduction factor (TF), currently in a bio- pharmaceutical development pipeline. A subset of AML and MDS has been found to have RARalpha pathway activation characterized by a large enhancer at the RARA locus (RARA-high) and/or upregulation of IRF8, a TF associated with RARA signaling, forming the basis of a new PD-sensitive tumor identification.

Aims: We sought to understand how SY-1425 agonism of RARalpha acts to promote maturation and halt proliferation of AML blasts locked into an immature cell state by the cancer circuity. This characterization could further inform clinical pharmacodynamics markers.

Methods: We analyzed the epigenomic and transcriptional landscape of 66 non-APL AML patients and normal primary myeloid cells by RNA-seq and ChIP-seq for the enhancer marker H3K27ac. AML cell lines were profiled by RNA-seq, ChIP-seq for H3K27ac and RARalpha, and ATAC-seq with or without SY-1425 treatment. Cell surface marker changes were assessed by flow cytometry. Results: A subgroup of the patient samples was defined by an SE driving RARA, which co-occurred with SEs driving FOS and JUNB, or IRF8, FOS and JUNB form the AP-1 heterodimeric TF known to promote an immature cell state and the interferon regulatory factor 8 (IRF8) pathway has been implicated in AML pathogenesis. Previously reported crosstalk between INF and retinoic acid signaling was supported by the strong induction of interferon gene sets by SY-1425 in IRF8-high AML models. We found that each AML cell line had distinct compositions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocyctic, macrophage, dendritic, and granulocytic cell types. While AML has a broad and diversified and IRF8 and granulocytic differentiation, we found that RARalpha/IRF8-high AML could follow multiple differentiation paths depending on the initial state of the AML model, necessitating different marker panels to capture full cell typing. Functional validation confirmed surface marker changes consistent with RARalpha/IRF8-high AML. We observed epigenetic alterations including ID11b, CD11c, CD66b, and CD38 upregulation. We integrated epigenetic data, DNA accessibility, and SY-1425 response to understand RARalpha perturbation to cell circuity. Enhancer elements directly bound by RARalpha were associated with greater response to SY-1425 as was enhancers bound by other TFs involved in myeloid differentiation. The accessibility of RARalpha and IRF8 motifs were increased and their associated TFs were upregulated. The target genes of known immu- norepressive state drivers, such as RUNX1 and CEBP, were downregulated. Importantly, the FOS/JUN circuit, identified as a component of the oncogenic RARalpha circuit in patient samples, was found to be suppressed.

Results: Genomic and functional analysis identified RARalpha and associated cell state TFs play a critical role in the differentiation of AML. SY-1425 perturb- ation of this circuity leads to differentiation toward multiple potential lineage paths depending on the initial state of the cancer. These pharmacodynamic changes can be assessed clinically and combined with common AML/MDS markers to assess disease burden and develop therapeutic strategies for t-AML. Finally, because it has been proposed that t-AML might be more prone to clinical and preclinical data to inform current and future clinical studies.

E885
GENETIC CHARACTERIZATION OF A LARGE GROUP OF CBESA MUTATED AML PATIENTS AND THE EFFECT OF TET2 AND GATA2 MUTATIONS ON OUTCOME
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Background: Mutations in the CEBPA gene are detected in about 10% of patients (pts) with cytogenetically normal (CN) acute myeloid leukemia (AML). CEBPA mutations can either be biallelic (bi) or monoallelic (mo). Only pts with bi-CEBPA mutations have favorable outcomes when compared to other CN-AML pts. bi-CEBPA mutations are rarely associated with other prognostic mutations like internal tandem duplications (ITD) or mutations in the tyrosine kinase domain (TKD) of the FLT3 gene or mutations in NPM1. There is a specific association of bi-CEBPA mutations with mutations in the transcription factor GATA2.

Aims: In this study we aimed to characterize the mutational spectrum of CN-AML pts with mo- and bi-CEBPA mutations. We further analyzed the effect of TET2 and GATA2 mutations on outcome in pts with bi-CEBPA mutations.

Methods: Targeted amplicon resequencing (Agilent Haloplex, target region: 63kb) was used to analyze 42 target genes or hotspots known to be mutated in CN-AML or other hematologic neoplasms.

Results: In 48 bi-CEBPA and 32 mo-CEBPA we found mutations in 20 and 26 different genes respectively. Mo-CEBPA pts showed significantly more additional mutations compared to bi-CEBPA pts (mean: 3.9±1.7 vs 2.2±1.5; p<0.001). We also compared the mutational profile of mo- and bi-CEBPA pts with a collection of 106 CN-AML pts. bi-CEBPA pts had a higher occurrence of CN-ITD (21.6%) vs a lower occurrence of CN-ITD (11.4%) in mo-CEBPA pts. bi-CEBPA mutations were significantly more frequent in bi-CEBPA pts (21.2%) vs mo-CEBPA pts (12.5%; p<0.001). FLT3-ITD1/TKD2 mutation frequency in bi-CEBPA significantly differed from mo-CEBPA (p=0.002) and wt-CEBPA (p=0.004). There was a significant difference in the frequency of FLT3-ITD in bi-CEBPA (20.8%) vs wt-CEBPA (4.5%) and mo-CEBPA (0.0%) but not in comparison to mo-CEBPA (43.8%). IDH2 was found mutated only in wt-CEBPA (21.6%) and mo-CEBPA (18.8%). In 48.5% of wt-CEBPA pts DNMT3A was mutated, this significantly differs from bi-CEBPA pts (14.3%; p<0.001) but not from mo-CEBPA patients (28.1%). CSF3R was frequently mutated only in bi-CEBPA (10.4%) but not in mo-CEBPA (0.35%; p<0.001) or mo-CEBPA (3.1%; ns). STA2G was associated with mo-CEBPA (25%), while STAG2 mutations were significantly less frequent in bi-CEBPA (6.3%; p<0.001) and wt-CEBPA pts (6.27%; p=0.002). TET2 mutations had a negative prognostic impact on overall survival (OS) in bi-CEBPA pts, but not in mo-CEBPA pts or in wt-CEBPA pts (p=0.033). CEBPA mutations were significantly worse in bi-CEBPA pts with a TET2 mutation, but relieve a poor outcome (RFS) and cumulative incidence of relapse (CIR) was not different depending on TET2 mutational status. In bi-CEBPA pts we also evaluated the clinical impact of GATA2 mutations. For 30 of 48 bi-CEBPA pts survival data was available, 15 of these pts were GATA2mut and 15 were GATA2wt. We observed a significant difference with respect to RFS (p=0.216), OS (p=0.479) and CIR (p=0.059). 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: bi-CEBPA, mo-CEBPA or wt-CEBPA pts show a distinct profile of co-occurring mutations that might explain the biological differences between these groups. TET2 mutations were found in 40% of all CEBPA mutated pts and might have a prognostic impact in bi-CEBPA pts.
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 10360x. Eighteen patients remained wild-type for all analyzed genes (Figure 1). Only one 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 832. 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.

<table>
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Summary/Conclusions: In summary, the present study shows that the mutation status of NRAS and FLT3 genes could be used as genetic markers for prognosis in 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 change in post-remission therapy.

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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Unfavorable recurrent mutations with PD-L1 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 using qRT-PCR method. For miRNA analysis, CD33+ cells from 29 AML patients were isolated and CD33+ miRNA expression was analyzed.

Results: We observed significant differences in PD-L1 expression in groups of 54 AML, 62 MDS and 8 s-AML patients compared to HVs. TCGA data analysis showed that high expression of PD-L1 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-1.16. Genotype AA was 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 observed significant differences in PD-L1 expression level regarding to PD-1.15 polymorphism. Moreover, analysis of a PD-1.13 polymorphism in HVs and MDS groups revealed that genotype GG 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-1.16. Genotype AA was associated with higher risk of shorter OS compared to the rest of the genotypes (58 vs 333 days, HR=35; p<0.001).

This work was supported by National Centre for Science Grant HARMONIA (UMO-2013/10/M/NZ5/00313).

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 divergent fitness provided by the often complex landscape, resulting intratumor heterogeneity, which is associated with extremely heterogenous and dynamic tumor cell population that can drive disease under many conditions. The relapse of human acute myeloid leukemia (AML) is a prime clinical example of how evolving sub-clonal dynamics can frequently drive treatment-resistant cancer recurrence after initially potent therapy.

Aims: We aimed to understand how single-lineage interference is regulated in AML in response to standard and emerging treatments - and clarify how this impacts the development of therapy resistance. Specifically, we aimed to dissect if relapse from each drug regimen was driven by predetermined or stochastically selected sub-lineages and determine the functional impact of such differences.

Methods: We dissected the intra-tumor population dynamics of relapsing AML, beyond the genetic level, by performing single-cell lineage-tracing through cellular barcoding technology (lentivirus-integrated non-coding DNA-tags). We
E890

Abstract withdrawn.

E891

MRD ANALYSIS BY NEXT-GENERATION SEQUENCING APPROACH FOR ACUTE MYELOID LEUKEMIA FOLLOW-UP
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Background: Sensitive detection of molecular marker of minimal residual disease (MRD) in acute myeloid leukemia (AML) could improve prognostication of a possible relapse during the remission. Traditional methods for measuring minimal residual disease (MRD) in AML, such as real-time PCR and multiparametric flow cytometry (MFC) are associated with high technical complexity, low applicability and laborious standardization. However, some patients who achieve a negative MRD become to relapse and several MRD-positive patients have a long survival, which indicates that the sensitivity and specificity of traditional techniques are not enough to detect the residual disease. Aims: To detect minimal residual disease in AML follow-up sample using high-throughput sequencing as a standard and accurate technique.

Methods: We studied 54 gDNA bone marrow follow-up samples (27 after induction, 10 after first consolidation, 17 after second consolidation) from 30 AML patients treated according to PHEMA AML clinical protocols and with DNA sample at diagnosis. All patients had achieve CR at the moment of MRD assessment. We developed a custom-targeted sequencing panel of 32 genes (ion Torrent Proton System-Thermo Fisher) for mutation (SNV and/or InDels) detection at diagnosis sample. From the 32 genes, we use specific primers to amplify the specific region of the four most frequent alterations at diagnosis (Samples at follow-up: FLT3n-ITD n=2, NPM1n=46, IDH2 n=9 or IDH1 n=7). We analysed and detected at diagnosis and at follow-up (after induction, first consolidation or second consolidation), and sequenced with high-throughput approach. We achieve a technical sensibility around 10-4 for point mutations and 10-5 for Indels mutations according to specificities and sensitivity calibration curves.

Results: We analysed the results of assessing MRD by NGS, and the presence or absence of MRD was established at a cut-off level of 0.0017 (between 10-4 and 10-5) with ROC curve with a sensitivity of 0.5 for DFS and 0.571 for OS, and a specificity of 0.92 for DFS and 0.897 for OS; thereby all result above this level was considered as MRD positive. DFS (Disease Free Survival) and OS (Overall Survival) rates in this group were 29.9% and 24.1%, respectively; positive MRD sample was independent marker associated with shorter DFS (p=0.002, HR=0.33, 95% CI:1.60-33.51) and OS (p=0.002, HR=8.33, 95% CI:1.87-37.15) (see figure 1). These results support the usefulness of MRD evaluation in patients with AML by NGS in the context of molecular biology studies.

Figure 1.

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.

This study was funded by Instituto Carlos III (PI13/02387).

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 without/with phorboxomaglutinin A10g/mL. T-cell proliferation was measured by carboxyfluoreescin 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 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, monocyte like subgroup 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 11.1%, 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 23.4%±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).

366 | haematologica | 2017; 102(s2)
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

GENERALIZATION 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 leukemia (AMKL) is a rare and complex type of Acute Myeloid Leukemia (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 preincidence and early human hematopoiesis, we postulate that they preserve their pluripotency and could serve as platforms to mimic human embryonic hematopoietic development. In this project, we aim to human hPSCs expressing non-DS AMKL-associated fusion oncomers as cellular models for this leukemia, 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-JARID1 using transduction with lentiviral vectors. 2. Generation of hPSCs with the chromosomal translocations t(1;22) and t(1;12), that generate the fusion proteins RBM15-MKL1 and NUP98-JARID1 respectively, and the inversion of chromosome 16, that originates the fusion protein CBFA2T3-GLIS2. 

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 leukemia occur in the developing fetus, we propose that human embryonic stem 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 oncomers as cellular models for this leukemia, to study the molecular and cellular pathways involved in the development of pediatric non-DS-AMKL.

Summary/Conclusions: These models will serve as platforms to discover and understanding the cellular and molecular alterations caused by these oncomers, 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: Hematologic malignancies can be driven by a diversity of mutation types, including single nucleotide variants, copy number variants, gene fusions, insertions and deletions and changes in gene expression profiles. However, comprehensive detection of these mutation types is challenging, as specific assays are required to detect each mutation type. Next-generation sequencing (NGS) enables comprehensive detection of all mutation types from whole genomes and transcriptomes. However, low detection sensitivity, high input requirements and high costs render these approaches impractical for routine detection of mutations from clinical sample types. Anchored Multiplex PCR (AMP™) is a target enrichment strategy for NGS that uses molecular barcoded (MBC) adapters and unidirectional gene-specific primers (GSPs) for amplification.

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 of several models and common hematologic malignancies. In particular, we sought to develop methods to detect novel fusion genes, internal tandem duplications (ITDs) and mutations in CEBPα.

Methods: We developed AMP-based Archer™ VariantPlex™ and FusionPlex™ assays to enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification enables detection 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 AXL-RUNX1, RUNX1-TAL1 and AML1-RUNX1 in acute myeloid leukemia and high expression levels relevant in hematologic malignancies. Importantly, AMP enables identification of known and novel gene fusions at nucleotide resolution, detection of ITDs and characterization of relative gene expression levels and CNAs.

Background: Mutations of ASXL1 are considered early founder events in AML and leukemogenesis. They are included in the definition of the “chromatin-splice-some” genomic class of AML and among the high risk genetic prognosticators in the 2017 ELN recommendations.

Aims: We aimed to study the frequency of ASXL1 mutations in a cohort of newly diagnosed AML patients and to look for correlations with conventional cytogenetic findings and baseline characteristics.

Methods: Three hundred and sixty AML patients diagnosed between 2005 and 2014 were studied. Conventional cytogenetic analysis was performed on unstimulated bone marrow cells cultured for 24 and 48 hours. Molecular analysis of ASXL1 exon 12 mutations was performed by PCR and subsequent direct sequencing in diagnostic bone marrow or peripheral blood samples.

Results: Median age of the whole cohort was 63 years (11-95) and 56% of patients were male. Eighty two patients (22.8%) had secondary AML (sec-AML) with prior diseases being MDS (63), CMML (4), PV/ET (9), MF (2) and CML (4). Karyotypic analysis was successful in 352 (97.7%) AML samples of which 104 (29.4%) exhibited clonal karyotypic abnormalities. ASXL1 mutations were detected in 52 patients (14.4%). The most common mutation was c.1934dupG in 44/52 (84.6%). ASXL1 mutated patients were significantly older with median age 72 vs 61.5 years in the unmutated (p=0.0001). Three of 61 patients (4.9%) aged ≥60, 10/97 aged 41-60 (10.3%) and 39/198 aged ≥60 (19.7%) were mutated. Avs. 11.5% vs 16.8%, p=0.07). ASXL1 mutations were significantly more frequent among cases with trisomy 8 (25% vs 12.8%, p=0.02) and patients with chromosome 11 abnormalities (23.7% vs 13.7%, p=0.01). None of the 12 patients with inv(16)(t(16;16)) was mutated while 2/16 (12.5%) of those with t(8;21) had ASXL1

haematologica | 2017;102(s2) | 367
mutations. Moreover, ASXL1 mutations were detected in 3 of 12 patients with aberrations detected (25%), 2/9 (22%) with trisomy 13, 2/11 (18%) with t(6;9) and only 1 of 22 patients with t(15;17). Multivariate logistic regression suggested 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 t(7;17) or -5/del5q 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 cyto-independend predictor. Despite the strong link with secondary AML (majority of cases post MDS), our data show inverse relationship with -7/del(7q) or -5/del5q. In addition, ASXL1 mutations were not positively associated with MDS-related cytogenetic abnormalities, complex or monosomal karyotypes.

E896
Abstract withdrawn.

E897
A COMPREHENSIVE DNA TEST FOR THE DETECTION OF TRANSLOCATIONS IN ACUTE LEUKEMIA

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1Genetics, 2Genetic Coordination Center, UMCG, Groningen, 3Cergentis B.V., Utrecht, 4Laboratory Medicine, 5Hematology, UMCG, Groningen, Netherlands

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(16;11), t(11;19)(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 with 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 suspected of chromosomal translocation were taken for routine diagnostic karyotyping (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 ALL samples, not detected with karyotyping but RT-PCR confirming the TLA findings. In fifteen samples no translocation was detected. In 15% samples t(6;9) was the only translocation detected in a patient with myelodysplastic syndrome. TLA testing was not performed 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(12;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 NECROTOPSIS 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 is younger 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 month vs 6 month respectively (p<0.001). With Fisher Exact Test we further demonstrate that copy number loss of RIPK1 or MLKL are associated to loss of TP53 or FANCA genes, complex karyotype and advanced age. COX-Hazard Ratio model with RIMK1 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.

Acknowledgment: ELN,AIL, AIRC, progetto Regione-Università 2010-12, FP7 NGS-PTL project,HARMONY.
E899

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 to diagnostic.

Aims: To evaluate the predictive impact of the fluctuation Variant Allelic Frequency in resistance to treatment cases in AML.

Results: Mutations in signalling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) show VAF trend decreases. No correlation was found between VAF and% blasts, nor did VAF fluctuation with blasts fluctuation.

Summary/Conclusions: These results show VAF increases of specific mutations as RUNX1 correlates with primary refractoriness status. Furthermore, the variable frequency signalling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) play a critical role in resistance status, increasing variant allelic frequencies of mutations in relapse and decreasing in refractoriness.

This work was supported by the grant: PI13/02387.

E900

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 phosphoproteomics 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/WT) 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 CalcuSoft 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 CalcuSoft software and normalized isobolograms were represented. All combinations showed CI values less than 0.5 (CI<0.5). The inhibition levels of four pathways (PI3K, STAT5, ERK1/2 and MAPK14) implicated in TKIs resistance were studied by western blot and the combination of midostaurin plus trametinib was the only one that inhibited all of them. Trametinib efficacy in the MOLM13 sorafenib-resistant culture was evaluated and confirmed that remained effective. Trametinib plus midostaurin combination was tested in OCI AML-3 cell line (FLT3WT/WT) showing high efficacy and strong synergy (CI<0.5) too. Finally, we have assayed these drugs ex vivo in three AML samples showing the same effectiveness as in vitro, with IC50 values ranging from 0.2µM to 0.9 µM for midostaurin and 3 nM to 29 nM for trametinib and CI values less than 0.5.

Summary/Conclusions: In conclusion, we provide preclinical evidence that combining 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.
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 chemotheraphy-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, DragQ 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 cell death in normal myeloid precursor cells derived from blood donors, normal cord blood, and pediatric AML patient cells representing distinct AML subtypes.

Results: From the 80 epigenetic compounds tested in THP-1, Kasumi-1 and CMK cells, we observed significant effects following treatment with the HDAC 4/5 inhibitor LMK235, the pan-HDAC inhibitor NSC3852, and the pan-bromodomain inhibitor Bromosporine. 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-1 and THP-1, resp. While inhibition by LMK235 resulted in an immediate response of apoptosis, Bromosporine-treated cells retained in G1 between 48 and 96 h, and, interestingly, LMK235 treatment resulted in 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 treatment with LMK235, 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 lineage-specific 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 valproic acid (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 IFNa-2b and human IFNa-Le, in relevance to AML treatment.

Methods: Flow cytometry and Hoechst staining was used to investigate apoptotic potential of the IFNa therapeutics, whilst phospho-flow cytometry and difference gel electrophoresis in combination with mass spectrometry unraveled IFNa signaling pathways. For in vivo efficacy analyses two orthotopic rodent models were implanted with leukemic cells and treated with VPA, IFNa-Le or both drugs.

Results: To investigate the anti-leukemic effects of IFNas 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 IFNa-Le was more effective compared to IFNa-2b in inducing apoptosis, whilst both drugs were ineffective in combination with VPA. Investigating IFNa signaling pathways using phospho-specific flow cytometry we found IFNa-2b and IFNa-Le to have an identical stimulation profile in MOLM-13 cells, except from p-STAT5 Y691 that was higher expressed by IFNa-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, HSP90AB1) and cell death (PKM2, PARK7, HSPA5, ANXA5, PRDX2) to be differently regulated between IFNa-2b and IFNa-Le, and also identified the presence of a dose-dependent effect of protein expression by IFNa-2b and IFNa-Le. Further, we investigated the potential synergetic anti-leukemic effects of VPA and IFNa-Le in vivo using a MOLM-13Luc+immunodeficient NOD/Scid IL2 g-/- orthotopic xenograft mouse model, and the expression of key anti-apoptotic proteins via CDK9/ RNA polymerase II, including MCL-1. With this understanding, we reasoned that alvocidib would enhance the activity of the 7+3 regimen.

Aims: These studies sought to interrogate the preclinical activity of alvocidib in the context of the 7+3 regimen in models for AML.

Methods: CellTiter-Glo and Caspase-Glo was used for all cell viability and apoptotic analyses, respectively, in the 7+3 model. Following manufacturer’s protocol. We used RT-PCR to measure mRNA expression of MCL-1 and other markers in response to drug treatment. Protein levels were interrogated using standard immunoblotting techniques. To determine the efficacy of an alvocidib/7+3 combination on tumor growth in vivo, we performed a MV4-11 xenograft mouse model. Using flow cytometry we found IFNa-2b and IFNa-Le to have an identical stimulation profile in MOLM-13 cells, except from p-STAT5 Y691 that was higher expressed by IFNa-2b. The phospho-proteome was further explored using difference gel electrophoresis in combination with mass spectrometry unraveled IFNa signaling pathways. For in vivo efficacy analyses two orthotopic rodent models were implanted with leukemic cells and treated with VPA, IFNa-Le or both drugs.

Results: Single agent IC50 values of alvocidib, cytarabine, and daunorubicin range in AML cell lines from 2.2 nM to as high as 567 nM in viability assays. In apoptosis (Caspase-Glo) assays, however, we observed modest induction with single agent cytarabine, and good induction with single agent daunorubicin or alvocidib. In the combination setting, we observed a strong synergy with more than two-fold enhanced induction of apoptosis in some treatment groups. As has been previously described, we report here too that alvocidib treatment reduced the expression of MCL-1 protein and mRNA in a time and concentration-dependent fashion in AML cells. We observed this in the 7+3 treatment as well. In an MV4-11 xenograft model, we observed 21.1 and 48.5% tumor growth inhibition (%TGI) following single agent treatment of daunorubicin or cytarabine, respectively. 1.25mg/kg alvocidib yielded 60.0% TGI. The combination of alvocidib, cytarabine, and daunorubicin, however, resulted in tumor regression, yielding a 116.2% TGI.

Conclusions: These results provide a clear rationale for a clinical study directly comparing the triple combination to 7+3 alone. Taken together, our results suggest that a combination of alvocidib, cytarabine, and daunorubicin might be a potential clinical regimen in treating frontline AML, offering patients additional treatment options in treating their disease.
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-13\textsuperscript{ace} mouse model, and from 21 days to 50 days in the BNML rat model. Additionally, the IFNα-Le (0.8x10\textsuperscript{6} IU/kg) and VPA (400mg/kg) combination treatment indicated a tendency to increased survival in the BNML model. However, IFNα-Le monotherapy (1x10\textsuperscript{6} IU/kg) decreased survival in the MOLM-13\textsuperscript{ace} model.

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 angiogenesis. 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 Celleceutix, 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 Celleceutix, 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 TP53 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 drop of mitochondrial membrane potential and a remarkable increment of SIRT-3, that cannot exert its inhibitory effect on p53. The MOLM-13 cell line showed a great p53 reduction, 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 60μg/ml.

Summary/Conclusions: IFNα does not add beneficial effects to VPA treatment in the two in vivo orthotopic models tested, possibly due to immune constitution and tumor load.

E905

CLEARANCE OF ‘DRIVER-COSMIC’ MUTATIONS POST CR1 WITH OPERATING RUNX1-L5ES IS UNLIKELY TO CONTRIBUTE TOWARDS DISEASE PROGRESSION IN AML

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Background: Clinical significance of gene variants in AML is well established (Papaemmanuil E et al, NEJM 2016) and is increasingly being implemented into routine diagnostic algorithms. Although 80% of patients achieve morphological remission after induction chemotherapy, long-term relapse free survival is a meagre 50% (Walter RB et al, JCO 2010. Monitoring of disease kinetics, is therefore, very critical.

Aims: To study the kinetics of gene variants post-induction chemotherapy in AML patients.

Methods: 130 follow-up samples from 45 de novo AML patients [median age-60 yr & median FU period- 18.6 mo] were screened for gene variants using TruSight Myeloid panel (Illumina CA) covering 54 genes with relevance in myeloid diseases. Gene variants at Variant allele frequency (VAF) of ≥10% at diagnosis and VAF of ≥15% during follow-up; both with target coverage of ≥300 reads were considered. Bone marrow (BM) or peripheral blood (PB) was obtained at presentation (BM-44; PB-1) and follow-up (BM-130). Gene variants in 95 samples from 40 MDS patients were also evaluated for progression to secondary AML. Public databases-Catalogue of Somatic Mutations In Cancer (COSMIC), dbSNP and 1000 genome (≥2%) were used to classify gene variants as either Drivers (D), variants of unknown significance (VUS) and germline polymorphisms (SNP). P-value was generated with 2-tailed Fisher Exact (GraphPad Software, Inc, USA).

Results: Of 45 AML patients 19 achieved complete morphological remission (CR), 21 had a relapse and 5 had refractory disease with a median of 4 mutations/patient in each subgroup. Driver mutation was identified in 38 patients; 82% of who had persistence until clinical end-point. While 17 of 18 relapse patients retained a driver only 9 of 15 patients in remission retained it (Table 1). 8 of the 9 patients had a ‘driver with COSMIC and SNP’ (D-C/S) reference that persisted, while all ‘driver with COSMIC only’ (D-C) disappeared post-induction. This suggests that drivers with both COSMIC and SNP reference may not always contribute towards disease progression. We also found that D-C mutations persist in 85.7% of relapse patients compared to only 11% of patients in remission (P-value: 0.001). Additionally, D-C mutations were retained in all 13 relapse patients with intermediate risk cytogenetics while complete clearance was observed in all 6 patients who were in sustained remission (P-value: 0.001). Further investigation of genes with D-C/S mutation in the remission cohort (8x) revealed that 4 patients had persistent DNM73A-25457242, 1 had DNM73A-25457243, 2 had RUNX1-36259324/L5ES and 1 had CBL-119149011. As DNM73A mutations are considered to occur in pre-leukemic
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.

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>Morphological remission</th>
<th>AML relapse</th>
<th>Refractory disease</th>
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<tr>
<td>No. of patients in each clinical outcome category</td>
<td>19</td>
<td>21</td>
<td>5</td>
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<tr>
<td>Mean Age (Range) (In years)</td>
<td>55.8 (32-71)</td>
<td>57.3 (30-77)</td>
<td>55.8 (31-83)</td>
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<td>No. of patients (years)</td>
<td>10 of 19</td>
<td>10 of 21</td>
<td>2 of 5</td>
</tr>
<tr>
<td>Time to clinical end-point (Range) (In months)</td>
<td>5.1 (3-15)</td>
<td>17.8 (9-44)</td>
<td>4 (1-13.22)</td>
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<table>
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<tr>
<th>Cytogenetics</th>
<th>Intermediate</th>
<th>Good</th>
<th>Poor</th>
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<tr>
<td>No. of patients with D-C mutation at presentation</td>
<td>12</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>No. of patients with D-C mutation that was observed until clinical end-point</td>
<td>9 of 15 (60%)</td>
<td>17 of 18 (94%)</td>
<td>5 of 10 (50%)</td>
</tr>
<tr>
<td>No. of patients who had D-C mutation and acquired new D-mutations at clinical end-point</td>
<td>0</td>
<td>8 of 17</td>
<td>0</td>
</tr>
<tr>
<td>No. of patients who lost D-C mutation and/or acquired new D-mutations at clinical end-point</td>
<td>6 of 15 (40%)</td>
<td>18 of 86 (21%)</td>
<td>0</td>
</tr>
<tr>
<td>No. of patients with clinical D-C mutation that disappeared and reappeared at clinical end-point</td>
<td>5 of 17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of patients without D-C mutation at presentation</td>
<td>40</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Of these how many acquired intermediate risk cytogenetics and D-C mutation at diagnosis</td>
<td>15 of 10</td>
<td>4 of 18</td>
<td>5 of 10</td>
</tr>
<tr>
<td>Of patients with intermediate risk cytogenetics and persistent D-C mutation</td>
<td>10 of 33</td>
<td>13 of 13 (100%)</td>
<td>1 of 100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>Morphological remission</th>
<th>AML relapse</th>
<th>Refractory disease</th>
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<tr>
<td>No. of patients in each clinical outcome category</td>
<td>19</td>
<td>21</td>
<td>5</td>
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<tr>
<td>Mean Age (Range) (In years)</td>
<td>55.8 (32-71)</td>
<td>57.3 (30-77)</td>
<td>55.8 (31-83)</td>
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<td>No. of patients (years)</td>
<td>10 of 19</td>
<td>10 of 21</td>
<td>2 of 5</td>
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<tr>
<td>Time to clinical end-point (Range) (In months)</td>
<td>5.1 (3-15)</td>
<td>17.8 (9-44)</td>
<td>4 (1-13.22)</td>
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<table>
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<th>Cytogenetics</th>
<th>Intermediate</th>
<th>Good</th>
<th>Poor</th>
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</thead>
<tbody>
<tr>
<td>No. of patients with D-C mutation at presentation</td>
<td>12</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>No. of patients with D-C mutation that was observed until clinical end-point</td>
<td>9 of 15 (60%)</td>
<td>17 of 18 (94%)</td>
<td>5 of 10 (50%)</td>
</tr>
<tr>
<td>No. of patients who had D-C mutation and acquired new D-mutations at clinical end-point</td>
<td>0</td>
<td>8 of 17</td>
<td>0</td>
</tr>
<tr>
<td>No. of patients who lost D-C mutation and/or acquired new D-mutations at clinical end-point</td>
<td>6 of 15 (40%)</td>
<td>18 of 86 (21%)</td>
<td>0</td>
</tr>
<tr>
<td>No. of patients with clinical D-C mutation that disappeared and reappeared at clinical end-point</td>
<td>5 of 17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of patients without D-C mutation at presentation</td>
<td>40</td>
<td>4</td>
<td>3</td>
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<tr>
<td>Of these how many acquired intermediate risk cytogenetics and D-C mutation at diagnosis</td>
<td>15 of 10</td>
<td>4 of 18</td>
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</tr>
<tr>
<td>Of patients with intermediate risk cytogenetics and persistent D-C mutation</td>
<td>10 of 33</td>
<td>13 of 13 (100%)</td>
<td>1 of 100%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Clearance of ‘Driver-COSMIC only’ mutations while RUNX1_L56S persists is unlikely to contribute towards disease progression in AML.

Summary/Conclusions: Our prognostic score based on simple and usual data: FLT3 status, cytogenic, 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

A. Wei1, A. Bajel2, N. Boiselay3, I. Kloos4, C. Delifer4, S. Banquet4, X. Thomas5, N. Vey6
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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 prosurvival 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 until for intensive chemotherapy (IC)), or MDS failing prior therapies.

Methods: A phase I 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 1300mg/day (median time on treatment: 43 days, range 1 to >374), 28 pts were R/R AML, 2 pts were elderly AML unfit for IC, and 4 pts had MDS failing prior therapies. Median age was 70 years (range 19-80), median of prior therapies 2 (range 0-6), median WBC 3.0 G/L (range 0-30). Among the AML cohort, European LeukemiaNet risk (Döhner 2010) was adverse in 53%, intermediate-I in 20%, and intermediate-II in 27%. Among the MDS cohort, 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 3.0 G/L (range 0-30). Among the AML cohort, European LeukemiaNet risk (Döhner 2010) was adverse in 53%, intermediate-I in 20%, and intermediate-II in 17%. Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. Most common (≥20% of pts) non-hematological adverse events (AEs), all grades, included diarrhea (27%), hypokalemia (27%), nausea (21%), and vomiting (21%). The most frequent grade ≥3 AEs were hematological (anemia (35%), thrombocytopenia (32%), febrile neutropenia (21%), and neutropenia (16%)), hypokalemia (18%), and sepsis (15%). Of 12 pts (38%) with AEs possibly related to study drug, the most frequent were diarrhea (3 pts), muscle spasms, thrombocytopenia, and anemia (2 pts each). One 74-year-old pt had grade 5 cardiac failure considered drug-related after 6 cycles of treatment (900mg). Non-related fatal AEs were reported (2 pts each). One 74-year-old pt had grade 5 cardiac failure considered drug-related after 6 cycles of treatment (900mg). Non-related fatal AEs were reported (2 pts each). One 74-year-old pt had grade 5 cardiac failure considered drug-related after 6 cycles of treatment (900mg). Non-related fatal AEs were reported (2 pts each). One 74-year-old pt had grade 5 cardiac failure considered drug-related after 6 cycles of treatment (900mg). Non-related fatal AEs were reported (2 pts each).

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.

DISSECTING THE CLINICAL HETEROGENEITY OF NUCLEOPHOSMIN-1 (NPM1) MUTATED ADULT ACUTE MYELOID LEUKEMIA: THE CONTRIBUTION OF FLOW-CYTOMETRIC DETERMINATION OF MINIMAL RESIDUAL DISEASE

E. Buccisano1,*, L. Maurillo1, M.I. Del Principe1, A. Di Veroli2, E. De Bellis2, L. Cicioni3, M. Divona3, T. Ottone3, S. Lavorgna3, V. Rossi4, A. Zizzari4, M.A. Imo Consalvo2, D. Fraboni1, C. Conti1, G. De Poeta1, M.T. Voso1, W. Arcese1, F. Lo Coco1, A. Venditti1
1Biomedicine and Prevention, University Tor Vergata, Rome, Italy

Background: Acute Myeloid Leukemia (AML) with mutations of the gene encoding Nucleophosmin-1 (NPM1) identifies a subgroup of patients with favorable prognosis according to the 2008 WHO classification. However, recent evidences (Papaemmanuel, NEJM 2016) suggest that the coexistence of additional gene mutations (e.g. DNM3TA, IDH1, IDH2R1408, and TET2) may determine an inferior clinical outcome as compared to favorable risk AML and precludes a reliable outcome prediction. The presence of minimal residual disease (MRD), as determined by quantization of NPM1 mutated transcripts, provides powerful prognostic information independent of other risk factors (Ivey, NEJM 2016).

Aims: The aim of our study was to investigate if detection of NPM1 by multi-parametric flow cytometry (MFC) might represent an alternative tool to discriminate different prognosis within the NPM1 mutated AML group, in a setting where an extensive gene profiling at diagnosis or a quantitative determination of NPM1 transcripts in remission would not be available.

Methods: We analyzed a series of 69 AML patients with NPM1 mutations; all the patients were in complete remission (CR) after intensive induction cycle of EORTC-GIMEMA protocols. The frequency of NPM1 mutated cases was not different among patients below (48/142, 34%) or above (21/61, 34%) the age of 60 years, respectively. Twenty out of 65 patients (31%) carried a concomitant FLT3-ITD mutation; 51/66 (77%) NPM1 mutated cases had a normal diploid karyotype. Upon full hematological recovery after consolidation cycle, counting, by MFC, ≥3.5×10^9 (0.03%) residual leukemic cells (RLCs) in the bone marrow (BM) was regarded as a condition of MRD positivity.

Results: Among NPM1 mutated patients, the rate of MRD negative CR was significantly lower (5/69, 7%) as compared to NPM1 WT ones (39/134, 29%), respectively (p<0.001). Although there was not a statistically significant difference, probably due to the low numbers, NMRD negative/NPM1 mutant patients had a lower Cumulative Incidence of Relapse (CIR) as compared to MRD negative/NPM1 WT ones (25% vs 60%). Also we evaluated the impact of autologous (AuSCT) or allogeneic (ASCT) transplantation on the outcome of MRD positive/NPM1 WT patients. The overall survival (OS) was significantly higher for patients submitted to ASCT (n=14) as compared to those (n=15) submitted to AuSCT (93% vs 33%, p<0.011). This was confirmed even after excluding from the analysis FLT3-ITD+patients. When all the meaningful clinical variables were challenged in multivariate analysis (MRD, type of transplant, age >60 yrs, karyotype), the type of transplant (ASCT vs AuSCT) was the only variable that significantly influenced OS and DFS (p=0.001 and 0.003, respectively).

Summary/Conclusions: In conclusion, although both quantitative RT-PCR represents the gold standard, MFC determination of MRD also confirms that the quality of remission is critical to discriminate patients with a different outcome among NPM1 mutant patients. In fact, these patients have a low chance to become MFC MRD negative and in a situation of MRD positivity, a very poor outcome can be substantially improved only by a timely use of an allogeneic procedure.

EXPRESSION OF IMMUNE CHECKPOINT MOLECULES (PD-1, PD-L1, PD-L2) ON BONE MARROW T CELLS IN ACUTE MYELOID LEUKEMIA

1Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, 2Department of Pediatrics, Asan Medical Center Children’s Hospital, University of Ulsan College of Medicine, 3Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic Of

Background: Immune checkpoints constitute a mechanism by which tumors escape from the host immune system and involve the programmed death-1 (PD-1) receptor and its ligands, PD-L1 and PD-L2. In a tumor microenvironment, the expression of PD-1, an inhibitory receptor on the surface of T cells, can lead to dysfunction of antitumor effector cells. Recently, investigators have detected overexpression of PD-1 for patients with acute myeloid leukemia (AML) who experienced relapse following allogeneic stem cell transplantation

Figure 1.
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 since 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 CD4+ T cells was significantly different compared with patients who experienced relapse after SCT (P=0.025 and P<0.0001), and NMTR after SCT (P<0.0001 and P<0.0001). 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<0.0001) or persistence (P<0.0001) after SCT were significantly higher than those of patients in the CTx groups. PD-1 and PD-2 expression on leukemic cells at time of diagnosis was higher in secondary AML transformed from myelodysplastic syndrome than in de novo AML (P<0.0001 and P=0.039). 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=0.038 and P=0.023).

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

C. Zhou1, H. Wei1, D. Lin1, Y. Wang1, B. Liu1, K. Liu1, B. Gong1, Y. Li1, G. Zhang1, S. Qiu1, R. Gu1, S. Wei1, X. Gong1, Y. Mi1, J. Wang1.*
Background: Currently, there is no consensus regarding optimal treatment for older patients with acute myeloid leukemia (AML). Decitabine for 5 consecutive days produced a complete remission (CR) rate of 17.8% in older patients with newly diagnosed acute myeloid leukemia (AML) treated with standard of care (SOC) chemotherapy, in pts with AML who receive indoximod in combination with 7+3 remission induction consisting of cytarabine (100mg/m^2/day for 7 days) and mitoxantrone. The overall response rate (OS) of AML was 20.7 months. One-year and two-year OS rates were 71.4% and 44.4%, respectively. Patients who responded to treatment had significantly longer OS than non-responders.

Summary/Conclusions: This indicates that the 10-day decitabine regimen may be an optimal management for older AML patients who are in intermediate or adverse cytogenetic risk group and fit for chemotherapy.

E912 INDOXIMOD IN COMBINATION WITH IDARUBICIN AND CYTARABINE FOR UPFRONT TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML): PHASE 1 REPORT
A. Emadi1,2,3,*, N.G. Holtzman1,2, M. Imran1, F. El Chaer1, M. Koka4, Z. Singh4, A. Shahlaee5, E. A. Sausville 1,2,3, J. Law 1,2, S. T. Lee 1,2, A. Banerjee 1,2, A. Rapoport1,2, M. R. Baer1,2, V. H. Duong1,2, D. H. Munn4, M. Loken2, E. Kennedy5, N. Vahanian5, C. Link6
1University of Maryland Greenebaum Comprehensive Cancer Center, 2Medicine, 3Pharmacology, 4Pathology, University of Maryland, Baltimore, 5Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Sciences, 6Link Genetics Co., Ames, United States

Background: AML cells can acquire immune evasion and tolerance through overexpression of the IDO (indoleamine 2,3-dioxygenase), which exerts immunomodulatory effects through tryptophan (Trp) catabolism and kynurenine production. By degrading Trp, IDO shifts the balance from a Trp-rich environment, which encourages T-cell proliferation and activation, to a Trp-poor environment. The common non-hematologic toxicities were febrile neutropenia and infections. Median overall survival (OS) of all patients was 20.7 months. One-year and two-year OS rates were 71.4% and 44.4%, respectively. Patients who responded to treatment had significantly longer OS than non-responders.

Summary/Conclusions: This indicates that the 10-day decitabine regimen may be an optimal management for older AML patients who are in intermediate or adverse cytogenetic risk group and fit for chemotherapy.

E913 PHASE III STUDY OF MEK INHIBITOR (MEK-162; BINEMITIN) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MYELOID MALIGNANCIES
K. Nadv1,2, T. Kadia1, G. Borthakur1, K. Takahashi1, P. Bose1, N. Daver1, Y. Alvarado1, M. Ohanian1, C. DiNardo1, E. Jabbour1, G. Garcia-Manero1, H. Kantarjian1, A. Patel1, F. Ravandi1
1Department of Leukemia, UTMD Anderson Cancer Center, Houston, United States

Background: Activation of the mitogen-activated protein kinase (MAPK) signaling (RAS/RAF/ERK/ERK pathway) promotes growth and inhibits apoptosis of hematopoietic cells. Inhibition of MEK/MEK/MAPK pathway has shown antiproliferative effects in acute myeloid leukemia (AML) and myelodysplasia (MDS). MEK-162 is an oral, potent, selective allosteric, ATP-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) of 40% 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 45mg twice daily was the first indecisive dose 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 female; 94% had a performance status of 1-2. Median number of prior therapies was 4 (1-6); 3/16 (19%) patients had complex karyotype. 11/16 (69%) patients were RAS mutated. 12/16 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 3.2 months (0.3-7.6). Common G3/4 toxicity included neutropenia (56%), fatigue (13%), nausea/vomiting (15%) 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
S. Clurea1, R. Saliba1, M. Shah1, S. Gaballa2, G. Rondoni1, J. Chen1, A. Gublis1, W. Wallis1, B. Orlan1, A. Alousi2, G. Bashir1, S. Ahmed1, D. Mann1, K. Rezvani1, S. L. Stroncek1, P. Khazaeli1, M. Gaudelush3, U. Popat1, J. Khouri1, C. Hosing1, P. Kebrina5, N. Daver3, M. Konopleva4, N. Parmar3, F. Ravandi1, J. Cortes1, H. Kantarjian1, R. Champlin1
1Stem Cell Transplantation, The University of Texas MD Anderson Cancer Center, Houston, 2Thomas Jefferson University, Philadelphia, 3Leukemia, The University of Texas MD Anderson Cancer Center, Houston, United States

Background: Acute myeloid leukemia (AML) is more common in the older population. Haploidentical stem cell transplantation (haploSCT) is a potentially curative therapy.
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 73%. Donors were children in 35 (81%) or siblings 10 (19%) patients.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>61 (55-69)</td>
</tr>
<tr>
<td>Disease</td>
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</tr>
<tr>
<td>AML</td>
<td>25 (58%)</td>
</tr>
<tr>
<td>MDS/AML MDS</td>
<td>8 (19%)</td>
</tr>
<tr>
<td>10 (22%)</td>
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</tr>
<tr>
<td>Follow-up</td>
<td>19 (6-49)</td>
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<tr>
<td>Cytogenetics</td>
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<tr>
<td>Poor</td>
<td>16 (37%)</td>
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<tr>
<td>Intermediate</td>
<td>54 (54%)</td>
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<tr>
<td>Good</td>
<td>31 (11%)</td>
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<td>RIC</td>
<td>29 (67%)</td>
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<tr>
<td>BM</td>
<td>42 (98%)</td>
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<tr>
<td>Disease Status</td>
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</tr>
<tr>
<td>CR1/2</td>
<td>22 (51%)</td>
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<tr>
<td>CR</td>
<td>3 (6%)</td>
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<tr>
<td>NRM</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>HCT-CI</td>
<td>Median 2 (range 0.1-11)</td>
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<td>Donors</td>
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<tr>
<td>Child</td>
<td>33 (8%)</td>
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<tr>
<td>Sibling</td>
<td>30 (63%)</td>
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<tr>
<td>Dose age</td>
<td>Median 37 (20-55)</td>
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<td>Sex mismatch</td>
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<tr>
<td>Female donors</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>Male recipient</td>
<td>10 (24%)</td>
</tr>
</tbody>
</table>

Figure 1.

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 1c). Thirty-five patients carried NPM1-mutation. Two-years OS for NPM1-mut patients showing more or less than 3.5 log transcript reduction at TP1 was 94, respectively (p = 0.039); 2 years OS for patients achieving NPM1-MRD negativity at TP2 was 90.5% vs 42.9% (p = 0.003). MFC MRD analysis in the NPM1-mut cohort led to results comparable with the whole cohort. Multivariate analysis showed that NPM1-MRD at TP1 was the strongest predictor for OS in this group.

Summary/Conclusions: Our data show that MRD assessment at different time-point reduction retains a strong prognostic impact in AML 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.

E915

OPTIMIZATION OF MINIMAL RESIDUAL DISEASE EVALUATION IN ACUTE MYELOID LEUKEMIA TO DRIVE POST INDUCTION THERAPY

P. Minetto1,*, F. Guolo1, M. Clavio1, M. Miglino1, A. Kunkl2, N. Colombo1, E. Fantini1, G. Fugazzola1, D. Guardo1, S. Matarrese1, F. Ballerini1, C. Di Grazia3, A.M. Raita2, A. Bacigalupo3, R.M. Lemoli1, M. Gobbi1

1Clinic of Hematology, Department of Internal Medicine (DIMI), University of Genoa, 2Service of Flow Cytometry, Department of Pathology, 3Division of Hematology and Bone Marrow transplantation, IRCCS AOI San Martino-IST, Genoa, Italy

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 received 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 MRD evaluation. MFC-MRD evaluation had been performed through 4-colour MFC analysis (and 5-colour from 2011). To define the MFC-MRD positivity cut-off values, were considered: of 2.5x10^-13 residuum leukemic cells (>0.025%) or a threshold of 1 x 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 was 94.1% and 91.5% at TP1 and TP2, respectively. 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 1a-c). However, patients with MFC-MRD >0.025 at TP1 displayed a heterogeneous outcome. In this subgroup WT1-MRD at TP1 was able to identify patients with the higher risk of death (Figure 1c). Thirty-five patients carried NPM1-mutation. Two-years OS for NPM1-mut patients showing more or less than 3.5 log transcript reduction at TP1 was 94, respectively (p = 0.039); 2 years OS for patients achieving NPM1-MRD negativity at TP2 was 90.5% vs 42.9% (p = 0.003). MFC MRD analysis in the NPM1-mut cohort led to results comparable with the whole cohort. Multivariate analysis showed that NPM1-MRD at TP1 was the strongest predictor for OS in this group.

Figure 1.

Summary/Conclusions: Our data show that MRD assessment at different time-point reduction retains a strong prognostic impact in AML 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.
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 stem 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 published prospective randomized controlled 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+ pro- genitor cell percentage in the bone marrow was analyzed. Platelet 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 neutrophilia 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 neutrophilia recovery after chemotherapy.

Summary/Conclusions: Our results indicate that CD34+CD38+CD117+HLA-DR+CD13+CD33+ cells before each course of chemotherapy is associated with chemotherapy and hematopoietic reconstitution capacity independently. These findings may help better understand endogenous hematopoietic reconstitution and modify future chemotherapy regimens based on progenitor cell percentages.

E918

MICRORNAS (MIRs) IN HIGH RISK PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) AS PREDICTION TOOLS FOR RELAPSE INCIDENCE AND RISK STRATIFICATION AFTER REJECTION OF ALLOLOGENIC STEM CELL TRANSPLANTATION

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Background: Despite recent progresses made in the treatment of acute myeloid leukemia (AML) (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 recurrence. Since microRNA (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. Method: 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) were studied. Informed consent has been obtained from either ND and RL samples, revealing 301 miRs that maintained their deregulation in the 2 subgroups, while 113 and 85 were uniquely found in ND and 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 the FLT3-ITD and MLL-rearranged sets respectively, underlying putting 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 (10;11)]. The identifications of new targets linked to this miRs would be useful in further studies focused on finding novel targeted based therapy. Interestingly, miR-34a-5p was recurrently found upregulated either in ND and RL groups, but not 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 anthracycline and cytarabine. The MRC 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. 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 first 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 28 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/sqm cumulative dose vs 8g/sqm). Among 8 intermediate risk patients who were MFC MRD negative post FLAI5 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 recurrence 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 these patients is characterized by shorter survival times and numerous 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 mitoxantron in relapsed and refractory AML (1, 2). Cladribine, 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 for 7 days in days 1-10 every 28 days for the remaining cycles of cladribine 5mg/m2 i.v on days 1-2 with LD-AraC (40mg/m2 s.c. 1-10 days). Responding patients were treated with a prolonged maintenance consisting of LD-AraC (40mg/m2 1-10 day). The treatment was continued until progression.

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. Time median number of cycles to obtain CR was 2 (range 1-3). 16% of patients do not responded to treatment. The regimen was well tolerated without 4-week and 8-week mortality. The main reason of death was: 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.

Figure 1.

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. Small panels (~10kb) are currently limited to the 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: 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 biotin, 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 conventional PCR with primers from both panels. 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.
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-genene panel using a series of contived 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/ITD detection from 0.5% to 25% is excellent (R² = 0.996 and 0.998, respectively). Average sequencing coverage was high, ranging from 5.26x to 7.68x. 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 the 2 methodologies. It showed 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 the WHO 2008 recommendations for AML diagnosis 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 US prospective, observational cohort study of patients with newly diagnosed AML (≥55 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 12 Dec 2013 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 academica vs community settings (76% [70/92] vs 62% [103/167], P = .018), normal vs abnormal karyotype (77% [79/102] vs 59% [79/133], P = .006), age <65 vs ≥65 (83% [85/103] vs 65% [80/123], P = .003), and white blood cell (WBC) count ≥20 × 10⁹/L vs <20 × 10⁹/L (81% [83/103] vs 74% [90/122], P = .025). In patients who had undergone molecular genetic testing (n=173), the mutations tested varied substantially. All of the NCCN-recommended molecular genetic tests were reported in 9% (15/167) of patients, corresponding 8% (670/8412) of patients 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 did not receive guideline-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 concentration-time curve (AUC) post-dose reductions in FLT3 positive pts; however, the identification of Q as the active metabolite, AC886, was a significant predictor of the QTC prolonging effect(Levis, et al. ASH 2016). Q and AC886 are both primarily metabolized by 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) aged 18–55 years (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. steady-state (SS) drug conc, following repeated once daily dosing, were predicted using non-parametric superposition. An analysis of variance (ANOVA) was performed to assess the CYP3A4 inhibitory effect of K and F on the PK.

**Results:** 93 HSs were enrolled (31 per arm). Inhibitors Q 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) C_max of Q by 17% and 11%, and GeoMean AUC_0-24 by 94% and 20%, respectively (Table 1 below). The GeoMean C_max and AUC_0-24 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 t_1/2 of Q and AC886 were 46% and 96% longer, respectively in Q+K vs Q+P. CL/F of Q was 17% lower and t_1/2 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 Q C_max and GeoMean AUC_0-24 was observed following repeat daily dosing of 30mg Q+K vs Q+P, 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.

**Table 1.**

**E926**

**CLINICAL OUTCOMES OF CHILDHOOD ACUTE MEGAKARYOBLASTIC LEUKEMIA: THE CHILDREN CANCER HOSPITAL EGYPT 57357 EXPERIENCE**

**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 median 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 patients had M7-0.1% 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 syndrome patients have poor outcomes irrespective of any cytogenetic abnormalities. Future direction to determining tumor biology based on molecular pathways in this disease is being considered.

**E927**

**IDENTIFICATION OF RESISTANCE ASSOCIATED CPG METHYLATION CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS UNDERGOING INDUCTION CHEMOTHERAPY**

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**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 azacitidine 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 azacitidine 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 azacitidine 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, whereas unsorted samples or day 0 and day 15 frequently clustered 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 seen in some of our unsorted 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 azacitidine prior to chemotherapy.

**Results:** In the Azacitidine 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 INS1M (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 ARID1 (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 EHF (ETS) (p=1e-226, 32.79% of 5752), CEBPE (p=1e-90, 10.34%), and Jun-AP1 (p=1e-4S, 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%).

A median overall survival did not differ between the two treatment groups, with 153 days for chemotherapy and 143 days for azacitidine plus chemotherapy patients.

**Summary/Conclusions:** Methylation changes associated with azacitidine and chemotherapy in 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 Azacitidine treatment more hypomethylated loci were observed. This potentially indicates DNA hypomethylation in vivo.

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**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 lncRNA namely ZEB2-AS1 would be associated with clinical outcomes, we assessed its expression in retrospectively collected paired samples of 10 of 16 AML patients.

**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 lncRNA by small interfering RNAs.

**Results:** Results showed that expression of ZEB2-AS1 lncRNA 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 lncRNA had significant shorter 3-year overall survival (OS) (0% vs 68.2%, p=0.036) and disease-free survival (DFS) (25.0% vs 69.8%, p=0.039). In addition, patients with higher expression of ZEB2-AS1 lncRNA 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 lncRNA, 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 26.6%, p=0.049) compared to that of chemotherapy.

**Summary/Conclusions:** Moreover, knockdown of ZEB2-AS1 lncRNA 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 lncRNA may serve as candidate to improve conventional risk stratification system and contribute to evaluating therapeutic responses. Furthermore, ZEB2-AS1 lncRNA could potentially be a therapeutic target to treat AML.
SALVAGE TREATMENT FOR ADVANCED ACUTE MYELOID LEUKEMIA

DECITABINE COMBINED WITH HAAG REGIMEN IS AN EFFECTIVE

Aims: To evaluate the clinical efficacy and safety of decitabine (DAC) in combination with HAAG regimen (HDAc, 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. Eighteen 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 22.2% (8/36) 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 4 hematological 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 relapsed 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.

E930

PROGNOSTIC IMPACT OF IDH1 AND IDH2 MUTATIONS IN LOW AND INTERMEDIATE RISK AML: A MULTICENTER RETROSPECTIVE STUDY

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 LeukemiaNet 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 25% of our patients. 7% were IDH1 R132, 16% were IDH2 R140 and 2% R172. Median WBC count was 12.66±10^9/L in IDH wild-type, and 24.71±10^9/L in IDH mutated. Absolute neutrophil count was 3.1±10^9/L in IDH wild-type and 0.8±10^9/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 in IDH mutated patients (overall 3+7 “like” regimens in 57 patients, FLAi like regimens in 42). There were not significant differences in CR rate after induction therapy, OS and PFS between IDH mutated and unmutated patients. Median OS was 595 days in IDH mutated and 467 in IDH wild-type (p=0.07). Median DFS was 319 days in IDH mutated and 406 days in unmutated (p=0.157). We further analyzed the impact of IDH mutation in different cytogenetic risk groups: OS and PFS were not significantly different between mutated and unmutated low cytogenetic risk patients. In intermediate I and II risk patients OS was significantly different (177 days in IDH mutated vs 406 days in IDH wild type, p=0.002), but OS was not. We then evaluated patients with normal karyotype and mutated NPM1. IDH mutations had no impact on OS and PFS.

Summary/Conclusions: In this multicenter retrospective study we found that lower absolute neutrophil count at diagnosis is significantly correlated with IDH mutation as already confirmed by other groups. In terms of prognosis we only demonstrated an advantage in DFS for unmutated intermediate risk patients, suggesting a negative prognostic impact of the mutations which need to be confirmed in further studies.

E931

DECITABINE COMBINED WITH HAAG REGIMEN IS AN EFFECTIVE SALVAGE TREATMENT FOR ADVANCED ACUTE MYELOID LEUKEMIA

Methods: We conducted a nationwide population-based cohort study and included all AML patients >25 years diagnosed in Denmark between 2000-2014 (follow-up ended Feb 2016). We compared chance of intensive chemotherapy, complete remission (CR) and chance of alloHSCT in CR1 by treatment-related mortality and marital status. Cox regression (HRs) were used to compare survival in acute myeloid leukemia (AML) patients using individualized socioeconomic and clinical data from Statistics Denmark and The Danish National Acute Leukemia Registry.

Figure 1.

Results: Median age for all patients was 60 years. IDH mutations were detected in 25% of our patients. 7% were IDH1 R132, 16% were IDH2 R140 and 2% R172. Median WBC count was 12.66±10^9/L in IDH wild-type, and 24.71±10^9/L in IDH mutated. Absolute neutrophil count was 3.1±10^9/L in IDH wild-type and 0.8±10^9/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 in IDH mutated patients (overall 3+7 “like” regimens in 57 patients, FLAi like regimens in 42). There were not significant differences in CR rate after induction therapy, OS and PFS between IDH mutated and unmutated patients. Median OS was 595 days in IDH mutated and 467 in IDH wild-type (p=0.07). Median DFS was 319 days in IDH mutated and 406 days in unmutated (p=0.157). We further analyzed the impact of IDH mutation in different cytogenetic risk groups: OS and PFS were not significantly different between mutated and unmutated low cytogenetic risk patients. In intermediate I and II risk patients OS was significantly different (177 days in IDH mutated vs 406 days in IDH wild type, p=0.002), but OS was not. We then evaluated patients with normal karyotype and mutated NPM1. IDH mutations had no impact on OS and PFS.

Summary/Conclusions: In this multicenter retrospective study we found that lower absolute neutrophil count at diagnosis is significantly correlated with IDH mutation as already confirmed by other groups. In terms of prognosis we only demonstrated an advantage in DFS for unmutated intermediate risk patients, suggesting a negative prognostic impact of the mutations which need to be confirmed in further studies.

E932

LESS-INTENSIVE TREATMENT LEADS TO DECREASED SURVIVAL IN UNMARRIED ACUTE MYELOID LEUKEMIA PATIENTS AND PATIENTS LIVING ALONE. A DANISH NATIONAL POPULATION-BASED COHORT STUDY

Background: Marital status has been found to affect leukemia survival. Still, lack of individual-level socioeconomic data, cohabitation status, and treatment information prevented further investigation of underlying mechanisms. As treatment is changing towards outpatient-care, effects of social support may become even more important.

Aims: We investigated whether and how cohabitation and marital status affect chance of intensive remission-induction chemotherapy and allogeneic stem cell transplantation (HSCT), treatment response, and survival in acute myeloid leukemia (AML) patients using individualized socioeconomic and clinical data from Statistics Denmark and The Danish National Acute Leukemia Registry.

Methods: We conducted a nationwide population-based cohort study and included all AML patients >25 years diagnosed in Denmark between 2000-2014 (follow-up ended Feb 2016). We compared chance of intensive chemotherapy, complete remission (CR) and chance of alloHSCT in CR1 by treatment-related mortality and marital status. Cox regression (HRs) were used to compare survival in acute myeloid leukemia (AML) patients using individualized socioeconomic and clinical data from Statistics Denmark and The Danish National Acute Leukemia Registry.
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 to 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 performance 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 OR 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 allogHCT 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 4/13 patients who did not achieve complete remission, 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 unmarried patients (never-married: adjusted HR 1.29 (CI=1.06-1.57), divorced/widowed: adjusted HR 1.11 (CI=1.00-1.23)) compared to married patients. In contrast, 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 cohabitation and marital status on 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 after 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 characterized by a favorable prognosis. Most patients achieve hematological complete remission (CR) and are not considered eligible for an early allogeneic stem cell transplantation (SCT). Nonetheless, the importance of minimal residual disease (MRD) monitoring in NPM-AML is well recognized but no data are currently available on MRD-directed therapy in this AML subset. Since 2004 we have prospectively evaluated NPM1 MRD at precise time points to evaluate salvage therapy.

Aims: To investigate the amount and clearance of MRD reduction and LAIP changes in de novo AML during chemotherapy and compare the results with clinical outcomes.

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+3x 2 induction and 2 consolidation. Among them favorable cytogenetics was in 4pts (t(16;21)-1, 16q22-1, t(8;21)-2pts), intermediate-7 (6-with normal caryotype, 1-t(17;22), poor-3 (complex karyotype-2, 11q23-1p3). Bone marrow samples were studied in standardized panel with most common antibodies by 6-color MFC (BD FACSCTo 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 assessed LAIP changes in patients with CMR 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-wt FLT3+, 1-NPM1+). 2 pts had resistant AML after 2 courses (DR). 3 pts out of 7 with complete morphological remission (CMR) after 1st course had MRD positivity (0.03%, 0.16%, 8.3%), and these pts became MRD-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 CMR and we diagnosed 1 morphological relapse (patient with MRD negativity and CMR after 2nd ChT). Two early relapses were also detected: both with persistent 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 CMR (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 CR after 2nd ChT and during ChT one of them gained CD56 and CD13, 2nd lost CD65 and CD11b, 3rd — gained CD56, 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 chemoresistant leukemia clone, followed by subsequent relapse.

Summary/Conclusions: The most favorable group consisted of MRD negative pts after 1st course. LAIP changes are common in pts with less favorable prognosis.

E935

LENALIDOMIDE MAINTENANCE IN PATIENTS WITH HIGH RISK ACUTE MYELOID LEUKEMIA

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Madrid, Spain, June 22 – 25, 2017 | haematologica | 2017; 102(s2) | 383

Figure 1.

Summary/Conclusions: Despite the good overall prognosis, a significant proportion 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 different researchers and it is an additional independent factor in clinical outcomes. The prognostic value of leukemia associated immunophenotype (LAIP) changes in not explored enough.

Aims: To investigate the amount and clearance of MRD reduction and LAIP changes in de novo AML during chemotherapy and compare the results with clinical outcomes.

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+3x 2 induction and 2 consolidation. Among them favorable cytogenetics was in 4pts (t(16;21)-1, 16q22-1, t(8;21)-2pts), intermediate-7 (6-with normal caryotype, 1-t(17;22), ppoor-3 (complex karyotype-2, 11q23-1p3). Bone marrow samples were studied in standardized panel with most common antibodies by 6-color MFC (BD FACSCTo 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 assessed LAIP changes in patients with CMR 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-wt FLT3+, 1-NPM1+). 2 pts had resistant AML after 2 courses (DR). 3 pts out of 7 with complete morphological remission (CMR) after 1st course had MRD positivity (0.03%, 0.16%, 8.3%), and these pts became MRD-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 CMR and we diagnosed 1 morphological relapse (patient with MRD negativity and CMR after 2nd ChT). Two early relapses were also detected: both with persistent 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 CMR (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 CR after 2nd ChT and during ChT one of them gained CD56 and CD13, 2nd lost CD65 and CD11b, 3rd — gained CD56, 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 chemoresistant leukemia clone, followed by subsequent relapse.

Summary/Conclusions: The most favorable group consisted of MRD negative 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.

Figure 1. 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), 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-heme toxicities 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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<tr>
<td>Age</td>
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<tr>
<td>WBC (10^9/L)</td>
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<tr>
<td>Platelets (10^12/L)</td>
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<tr>
<td>Creatinine</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 surpass 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 without HCT in patients ≤60 years or 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(31-3384) days. Early relapse rate (RR) and NRM were 11.0% 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 univariate 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 HCT, 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 IDAC, respectively, differences among groups were significant (p=0.001947 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 busulphan 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. 15-25809A. All rights reserved.

384 | haematologica | 2017; 102(s2)
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 therapy and follow up 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 (Knipp 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 25 years after publication of the initial study.

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 leukemia, only one patient died of another cause and only one single patient is still alive and well over a decade later.

E938

FLAG-IDA FOR RELAPSED/REFRACTORY ACUTE MYELOID LEUKAEMA: A SINGLE CENTRE 5-YEAR STUDY

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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 idarubicin) 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 the efficacy and safety profile of 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.

Figure 1.

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.

E939

A MULTICENTER, RETROSPECTIVE ANALYSIS OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA WHO WERE TREATED WITH DECITABINE

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Background: While acute myeloid leukemia (AML) is the disease of the elderly, treatment options has been limited for elderly patients. Decitabine is widely accepted as the treatment options for them. However, the efficacy has yet been evaluated in Asian population where difference of clinical manifestation or cytogentic had been noted.

Aims: In the current study, we conducted a multicenter, retrospective analysis on elderly AML patients from 8 tertiary institutes in Korea who were treated with decitabine in order to confirm whether the clinical outcomes of this agent are also acceptable in this population, and to provide further understanding of the disease nature of AML arisen in elderly patients.

Methods: Patients diagnosed with AML from 2013 to 2016 were included in the analysis. The inclusion criteria were as follows: (1) 65 or older patients with newly diagnosed, histologically confirmed AML (myeloid blast ≥20% either in the bone marrow or peripheral blood); (2) Treated with decitabine in a schedule of 20mg/m2 for five days every 4 weeks in patients. The primary end-point of the study was OS. We compared our data to the data from another Korean retrospective analysis, in which elderly patients with AML were treated with decitabine in order to confirm whether the clinical outcomes of this agent are also acceptable in this population, and to provide further understanding of the disease nature of AML arisen in elderly patients.

Results: A total of 80 patients were eligible for the analysis. The median age of patients was 74 years (range, 64 to 86 years) and 49 patients (61.3%) were male. Regarding the risk group, 6 (7.5%), 49 (61.2%), and 25 (31.3%) cases were classified as favorable, intermediate, and poor risk group, respectively. The patients had received median 3 (range 1-27) cycles of treatment and the median OS for all patients was 10.2 months. The median OS durations according to the cytogenic risk group are as follows: 12.4 months (95% CI 11.4-13.4) for favorable risk group (N=6), 13.6 months (95% CI 8.7-18.5) for intermediate risk group (N=49), and 5.5 months (95% CI 1.4-9.6) for poor risk group (N=25) (p<0.01). And when we categorized our cohort into two groups, that is, ECOG-PS 0-2 vs. ECOG-PS 3 & 4, those with good performance status demonstrated improved survival (11.5 months (95% CI 6.6-16.4) vs 4.4 months (95% CI 2.4-6.4), p=0.04). The OS curves according to prognostic factors are provided in figure 1. Next, we compared our data to another Korean retrospective analysis dealing with elderly AML patients who were treated with either best supportive care or intensive treatment. Although in our analysis more patients with poor performance status and elderly patients, it seems that outcomes of decitabine treatment are fairly better than that of best supportive care (OS 3 months) and comparable to intensive chemotherapy (12.1 months).
E940

DRUG-DRUG INTERACTION POTENTIAL OF GILTERITINIB IN HEALTHY SUBJECTS AND PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

Aims: To evaluate drug-drug interaction potential with gilteritinib in healthy subjects and patients with relapsed/refractory (R/R) AML.

Methods: The effects of CYP3A4 inhibitors (itraconazole [ITZ] and fluconazole [FLZ]), as well as a CYP3A4 inducer (rifampin [RIF]), on the gilteritinib pharmacokinetic (PK) profile were assessed in an open-label, parallel-group study conducted in 81 healthy subjects. Gilteritinib was administered as a single 10mg dose alone on Day 6, or in combination with 200mg ITZ administered twice daily on Day 1 and once daily on Days 2–28, or in combination with once daily 400mg FLZ on Day 1 and 200mg FLZ on Days 2–28. When given concomitantly with ITZ or FLZ, gilteritinib was administered on Day 6. In an additional cohort, RIF 600mg was administered on Days 1–21 and gilteritinib was administered as a single 20-mg dose on Day 8. Additionally, the potential inhibitory effects of gilteritinib on the PK profile of a CYP3A4 substrate (midazolam) were assessed in a cohort of patients with R/R AML (n=9) in the Phase 1/2 CHRYSLIS study (NCT02014458). Patients received oral gilteritinib (300mg/d) and single oral midazolam (2mg) doses. Gilteritinib was administered on Cycle 1 Day 1 and continued once daily in 28-day cycles; midazolam was administered on Day-1 and Cycle 1 Day 15. Furthermore, in patients with R/R AML, gilteritinib trough concentration data for patients on strong (eg, voriconazole or posaconazole) or moderate (eg, ITZ or FLZ) CYP3A inhibitors were compared with those for patients not using CYP3A4 inhibitors.

Results: In healthy subjects, gilteritinib exposure (expressed as C_max 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 C_max (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 CYP3A inhibitors (eg, ITZ or FLZ) may be permissible, precaution is warranted.

E941

A FLUDARABINE-BASED ACUTE MYELOID LEUKEMIA INDUCTION IS WELL TOLERATED UP TO 75Y OF AGE ALLOWS EARLY CONSOLIDATION AND LONG TERM SURVIVAL. A SINGLE CENTRE EXPERIENCE OF 136 CONSECUTIVE PATIENTS

Aims: We report a single center, real life experience of unselected 136 consecutive AML patients treated since 2002 in our center with Fludarabine, Aracytin, Idarubicin and or Etoposide: FLAIE up to 65ys or FLAI up to 75ys.

Methods: Patients were treated with the FLAIE or FLAI regimen followed by Idarubicin plus Aracytin 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 AraCy, autologous (Auto) or allogeneic (Allo) stem cell transplantation according to cytogenetic 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 (34/136) had strong, 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 therapeutic strategy was well tolerated to low SOX4 expression. The median overall survival was 9 months and factors significantly affecting OS were age below 50ys p<0.001; de novo AML p=0.003; good/intermediate CMR risk p<0.002; intensive consolidation with Allo or Auto transplant p<0.001 compared to chemotherapy alone. The mean probability of Leukemia free survival (LFS) was 88 months (median not reached). Patients above 50ys had a worse survival than patients younger than 50. The median OS and LFS rates 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=0.12) and median LFS was 15.9 and 23.6 months (p=0.71) respectively. Summary/Conclusions: In our real life experience the FLAIE/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 well tolerated to the patients with low SOX4 expression, because the low OS and LFS efficacy may be related to the high toxicity of intensive induction regimens and the nature of poor risk prognostic factors.

E942  
OVEREXPRESSION 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 AML1-ETO, NUP98-DDX10, and PML-RARα; the overexpression of HOXA9, CREB, and Evi1, 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 deregulation of Mef2c expression to induce myeloid leukemia in recipient mice. Sox4 gene was also reported to be 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 work 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 evaluate 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 test, and Chi-square test and the software used for multivariate 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=33) and high expression group (score 3, n=50), respectively. The variance of 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 risk of relapse compared to low SOX4 expression (19/36, 52% vs 13/49, 26.5%, P=0.028). Furthermore, with a median follow-up period of 46.7 months (range: 0.7 to 79.0 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=0.001, 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 (HR 1.924, 95% CI 1.020-3.628, P=0.043) irrespective of age, WBC count or FLT3/ITD status. For the 62 patients 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 BM 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 prognosis of AML patients.

E943  
AN OPEN-LABEL, MULTICENTER, PROSPECTIVE, RANDOMIZED STUDY OF RECOMBINANT HUMAN THROMBOPOIETIN AS AN ADJUNCT AFTER INTENSIVE CONSOLIDATION CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIA  
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Background: Thrombocytopenia is a common problem in the management of patients with acute myeloid leukemia (AML) receiving induction and consolidation therapy. AML patients with platelet count of less than 20×109/L might have a high risk of bleeding complications and had to take dose modifications instead of intensive chemotherapy leading to increased disease-free survival and overall survival. Platelet transfusions have a short therapeutic effect and are associated with all types of transfusion reactions. Recombinant human thrombopoietin (rhTPO) has been shown to improve the megakaryocyte and platelet development in solid tumor patients and immune thrombocytopenia (ITP) patients refractory to the conventional corticosteroids. We conducted this study to determine the availability of rhTPO in the platelet recovery after intensive consolidation chemotherapy with AML patients.  
Aims: The aims of this study were to identify the effectiveness and safety of rhTPO in supportive care in patients with AML receiving consolidation chemotherapy.  
Methods: Patients: Patients were eligible if they were 15-70 years of age who achieved complete remission after one course of IA induction therapy, and had platelet counts of less than 50×109/L after induction therapy, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0-3. Patients with AML M3 (acute promyelocytic leukemia) and AML M7 (acute myelomonocytic leukemia) were excluded from the study. All patients provided written informed consent according to protocol guidelines approved by the institutional review boards at their individual institutions. Study design: Patients received consolidation chemotherapy with DA, MA and intermediate-dose arabinosylcytosine (ARA-C), et al. When the platelet count was less than or equal to 50×109/L, patients in study group received 15000U/day of rhTPO (trade name: TIPOA) administration subcutaneously and patients in control group did not receive rhTPO therapy. The administration of rhTPO continued until the platelet count was more than 100×109/L or for the maximum of 21 days. Stanford University Baseline characteristics: There were no significant differences between samples test and chi-square test. Other statistical data analyses were performed using the two-tailed Student’s t test and were represented as means±SD of values. All differences were considered to be statistically significant when the P value was less than 0.05.  
Results: rhTPO did not result in hematologic effect was observed in the main characteristics between study group (n=49) and control group (n=36), including age, gender and other baseline characteristics. No patient withdrew. Platelet transfusion and time required for platelet recovery were shown in Table 1. Platelet transfusions: The mean number and days of platelet transfusions for the patients in the study group were significantly lower than those in the control group. There were no significant differences of statistic status between the patients. Platelet recovery: 1. rhTPO might reduce the duration of platelet count less than or equal to 20×109/L and 30×109/L after chemotherapy. 2. rhTPO could increase the maxim
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>Platelet Parameters</th>
<th>Study Group</th>
<th>Control Group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of platelets</td>
<td>7.82×10^11</td>
<td>7.82×10^11</td>
<td>0.154</td>
</tr>
<tr>
<td>Mean day of platelet count</td>
<td>2.0×10^11</td>
<td>2.0×10^11</td>
<td>0.154</td>
</tr>
<tr>
<td>Mean platelet count</td>
<td>4.2×10^11</td>
<td>4.2×10^11</td>
<td>0.154</td>
</tr>
</tbody>
</table>

Summary/Conclusions: rhTPO, administered as dose of 1500u/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 transusion 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 (in 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 6.85 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.

Table 1.

| Summary/Conclusions: | E945 SYSTEMATIC REVIEW OF HEALTH STATE UTILITY VALUES FOR ECONOMIC EVALUATION OF ACUTE MYELOID LEUKEMIA A. Foryythe1, V. Bal 2, M. Dolph 3, S. Patel 4, G. Tremblay5 1Purple Squirrel Economics, New York, 2Novartis Pharma, East Hanover, United States, 3Purple Squirrel Economics, Montreal, Canada, 4Novartis Pharmaceuticals UK Limited, Surrey, United Kingdom, 5Purple Squirrel Economics, Quebec city, Canada  

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 HUV using previously published algorithm by Crott et al. 2010, HSUV for induction, consolidation, cryoablation (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 OOL 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 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.
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+CRu, probably due to different impact of the second generation hypomethylating agent therapy. WBC >10000/µL as well as high cytogenetic risk. This apparent contradiction supports the idea that in elderly pts recovery of peripheral blood cells counts (PR+hematological improvement) is probably the most important factor influencing OS (Ferrara, Hemat 2016).

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. Hypomethylating 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 cytarabine in controlled randomized clinical studies (Kantarjian, JCO 2012; Cashen, JCO 2010).

Aims: In July 2016 we approved a retrospective and prospective multicentric observational 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 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 13/36 (23%) pts with WBCs>10000/µL. Cytogenetic analysis was performed in 52/56 pts, and in 24/43 (43%) molecular analysis including FLT3 and NPM1 mutations was performed. According to prognostic stratification, 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 9,5 months (range 4-19) and 4 months (range 1-15) in responder vs non responder pts. Table 1 shows response rate according to pts characteristics. At present time 18/22 alive pts are still on treatment with decitabine. Regarding toxicity, 23/36 (41%) pts manifested a grade ≥3 AEs although severe comorbidities (cardiovascular and metabolic) pre-existed in 14/23 (60,9%). A total of 35 hospitalization episodes due to toxicity were recorded and 10/56 pts (17,8%) died due to serious AEs. Overall the most common non-hematologic AEs were pneumonia and fever.

Table 1.

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+CRu, probably due to different impact of the second generation hypomethylating agent therapy. WBC >10000/µL as well as high cytogenetic risk. This apparent contradiction supports the idea that in elderly pts recovery of peripheral blood cells counts (PR+hematological improvement) is probably the most important factor influencing OS (Ferrara, Hemat 2016).

E947
ASPARAGINASE ERWINIA CHRYSAENTHEMI 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 adminis-tration 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 (RR) AML.

Methods: This was a phase 1, single-arm, pharmacokinetic investigator-initi-ated 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. Correlational 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 U/m², 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 20.1 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 28 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=0.031 for achieving partial remission (PR) and one achieved hematologic improvement (HI) after 6 doses of single agent Erwinaze. Both pts had plasma Gln levels <85 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).

Figure 1.
Summary/Conclusions: To the best of our knowledge, this is the first clinical report demonstrating that an asparaginase 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 asparaginase in AML, we are to investigate mechanistically-designed asparaginase 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 1.01 (0.32-2.54), 1.00 (0.45-5.73), 1.00 (0.19-2.83), 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 SOX* patients had shorter overall survival (OS) time compared to SOX0 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 seldom 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 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 patients with AML might 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 60mg/m²/day (N=15, 27.3%).

Results: Selected patient characteristics are summarized in Table1. Post-induction echocardiogram studies 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 stronger decrease in EF. So, the odds ratio for 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 MORBIDITY AND MORTALITY IN PATIENTS WITH INTERMEDIATE RISK ACUTE MYELOID LEUKEMIA - 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 from Houston Methodist Hospital was queried from the 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 risk groups. The intermediate cytogenetic group encompassed the majority of patients and offers unclear prognosis. We thus hypothesize the IWGMS scoring system can be utilized to divide the intermediate cytogenetic group into higher and lower mortality subgroups. In the intermediate cytogenetic group, high IWGMS score (>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 cytogenetic risk 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 the intermediate cytogenetic and low and higher mortality subgroups based on a combination of cytogenetic and genetic mutations. We expect similar results with data from other institutions.

Figure 1.

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 belong. We propose a systematic approach that integrates cytogenetic and 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.

E950

SUCCESSFUL IDENTIFICATION OF SPECIFIC AMINO ACID-DEPENDENCE IN ADULT T-CELL LEUKEMIA / LYMPHOMA (ATL) AND PRECLINICAL APPLICATION FOR NEW THERAPY

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Background: Adult T-cell leukemia / lymphoma (ATL) is highly aggressive malignancy caused by human T-cell leukemia virus type 1 (HTLV-1). As leukemia/lymphoma cells are often resistant to combination chemotherapy and recent antibody therapy, new strategies should be developed. Our laboratory recently found that proliferation and survival of hematopoietic stem cells are critically dependent on the amino acid valine (Science, 2016).

Aims: We here aimed to assess amino acid dependence of lymphoma and leukemic stem cells, and to establish a novel therapy by utilizing the differences in amino acid dependence between normal and leukemic stem cells.

Methods: First, primary ATL cells were sorted from samples of 7 typical acute-type ATL patients by 12-color flow cytometry, and serially passaged on stromal cells. Then passageable ATL cells from 3 patients were transduced with GFP-expressing lentivirus for tracking and counting by image cytometry. Using complete medium and twenty different culture media each lacking a single amino acid, we examined amino acid dependency of ATL cells. Amino acids vital for ATL cells were screened by co-culture with stromal cells. Effects of these media on normal lymphocytes of healthy volunteers were also examined. Finally, the effectiveness of amino acid restriction was evaluated in vivo by xenotransplantation of ATL cells into NOG mice. Mice were fed with different diets lacking specific amino acids at 6 weeks after transplantation, and sacrificed at 10 weeks for analysis of peripheral blood, organs, and lymphoma size.

Results: In vitro studies revealed that ATL cells have dependency on specific amino acids- cysteine, methionine, and valine. As 2-weeks restriction of the former two amino acids damaged stromal cells or normal lymphocytes, valine was picked up for further analysis. Proliferation of ATL cells was dramatically inhibited by valine restriction while the influence on normal cells was limited. Interestingly, valine restriction did not appear to affect the proliferative capacity of normal lymphocytes. Valine-depleted diet did not significantly reduce hemoglobin or platelet count, and there were no significant organ damages as far as examined macroscopically. Summary/Conclusions: We discovered that proliferation and survival of adult T-cell leukemia / lymphoma cells were dependent on valine. ATL cells could be eradicated by 4-weeks of valine in vitro. In-vivo model also showed that 4-weeks restriction of valine could dramatically reduce ATL tumor size. Valine-depleted diet did not significantly reduce hemoglobin or platelet count, and there were no significant organ damages as far as examined macroscopically.

Background: Angiogenesis (AG), with participation of the vascular endothelial growth factor (VEGF) and its receptor (VEGFR2), plays a key role in clinical features and outcome of patients with diffuse large B cell lymphoma (DLBCL).

The ability to induce AG is variable in humans, once that VEGF and VEGFR2 genes have several single nucleotide polymorphisms (SNPs) described with distinct proteins production. The wild-type alleles of VEGF -2578 C/A (rs999447) -2489 C/T (rs10922930) -1177 G/C (rs1570360), -534G/C (rs2010963), -460C/T (rs833081), 936C/T (rs3025039), and VEGFR2 -2710G/A (7667289) and -604T/C (rs2071559) SNPs determine higher production, transcriptional activity or binding efficiency of VEGF/VEGFR2.

E951

Aggressive Non-Hodgkin lymphoma - Clinical

VEGF AND VEGFR2 POLYMORPHISMS ARE INVOLVED IN AGGRESSIVENESS AND PROGNOSIS OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Angiogenesis (AG), with participation of the vascular endothelial growth factor (VEGF) and its receptor (VEGFR2), plays a key role in clinical features and outcome of patients with diffuse large B cell lymphoma (DLBCL).

The ability to induce AG is variable in humans, once that VEGF and VEGFR2 genes have several single nucleotide polymorphisms (SNPs) described with distinct proteins production. The wild-type alleles of VEGF -2578 C/A (rs999447) -2489 C/T (rs10922930) -1177 G/C (rs1570360), -534G/C (rs2010963), -460C/T (rs833081), 936C/T (rs3025039), and VEGFR2 -2710G/A (7667289) and -604T/C (rs2071559) SNPs determine higher production, transcriptional activity or binding efficiency of VEGF/VEGFR2.

Madrid, Spain, June 22 – 25, 2017

haematologica | 2017; 102(s2) | 391
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, Fish er’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 the cumulative incidence of event free survival (EFS) 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 hazard regression 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-type VEGF -1154G allele and VEGF-634G genotype were more common in stage III or IV patients. The wild-type VEGF -604GG 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 1154A and 936 T alleles had 1.52 and 1.52 more chances of presenting disease relapse 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 A/G SNP was associated with FFS and OS: patients with the variant VEGF 1154 A allele had 1.88 and 1.83 more chances of having an event.

Summary: Our data present, for the first time, preliminary evidence that inherited abnormalities in AG pathway, related to the wild-type VEGF -1154A allele and VEGF-634G genotype and VEGF-604G allele, influence critical features, response to R-CHOP and outcome of DLBCL patients.

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.

Methods: This is a retrospective multicenter study including patients older than 17 years, with a BMB and a PET/CT performed simultaneously as part of the routine pre-therapy staging for newly diagnosed DLBCL. Patients who had not received either chemotherapy or corticosteroids and no concomitant malignancy was known to be present at the time of both procedures. Only patients treated with R-CHOP as first line therapeutic strategy were included. Only variable was the obtained result in univariate analysis was included in the multivariate Cox regression for outcome predictors.

Results: A total of 271 DLBCL patients were initially identified; we excluded: 31 patients who received low intensity chemotherapy regimens (R-COP, Mini-CHOP-R, monotherapy with steroids) due to advanced age, comorbidities or detectable BM involvement; and DLBCL patients enrolled in clinical trials including standard regimens plus new agents (Bortezomib, Lenalidomide, Ibrutinib) or non-standard regimens (R-CHOP/14, Da-EPOCH-R, MACOP-B, Mega-CHOP, Hyper-CVAD). In the homogeneously treated R-CHOP(21)/205 DLBCL patients subset, the median age at diagnosis was 61 y.o. (range: 18-85), with a balanced gender distribution (51% female). Twenty-three of these patients (12.7%) had BMi on BM, whereas 43 (21%) had BMi according to PET/CT finding. Fifty-three patients (25.9%) had BMi according to either BM or PET/CT. Concordant BMi by means of both techniques was present in 16 (7.8%) patients. With a median follow-up of 25 months (15-47 months, p25-p75), 50 patients (24.4%) progressed or relapsed and 41 (20%) died. The 3-year estimated progression-free survival (PFS) and overall survival (OS) were 70%, and 78%, respectively. By univariate analysis, factors associated with a shorter PFS, with a <0.10, were: female gender, IPI3, abnormally elevated B2-microglobulin levels, PET/CT-BM(+) and BMi-BM(+) (+). In multivariate analysis only two factors were independent predictors of shorter PFS: VEGFR2 E954-634GG genotype and CD11b+CX3CR1+ monocytes. 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 1154A and 936 T alleles had 1.52 and 1.52 more chances of presenting disease relapse 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 A/G SNP was associated with FFS and OS: patients with the variant VEGF 1154 A allele had 1.88 and 1.83 more chances of having an event.

Summary/Conclusions: In our DLBCL cohort, treated with a uniform first-line chemotherapy regimen, BMi by BM complemented IPI in predicting those patients with a higher risk for relapse or progression, while IPI defined a subset of patients with a worse survival. In this cohort, BMi by PET/CT could not independently predict a shorter PFS and/or OS.
27.7 months (IQR, 14.6-46.1), 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-variable analyses demonstrated that age, ECOG performance status, B symptoms, extranodal involvement, NCCN-IPI, and PB-CD11b+CX3CR1+ cell group were significantly associated with OS. However, 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 IN-B-NHL, but not for high risk NCCN-IPI. In contrast, PB-CD11b+CX3CR1+ 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 used in differentiating 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) is unclear.

Aims: To characterize children with r-NHLS in AIEOP centers. Performing a retrospective analysis of r-NHLS AIEOP case records, describing main epidemiological, clinical and histopathological 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 neoplasias 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 analyzed. PB-CD11b+CX3CR1+ monocytes were under 40% (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-NHLSs; peripheral T-cell lymphoma (PTCL) n.o.s.; mycosis fungoides (MF); subcutaneous panniculitis T-cell lymphoma (SPTCL) and lymphomatoid papulosis (LP) amongst T-NHLSs; 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 ALC protocol, CHOP or CHOP and/or immunotherapy. Surgical resection has been performed in case of localized disease B-NHLSs only, followed by a W&S strategy, with 3-year OS and EFS were 76.5% and 60.9%, respectively. 3(5)/4110 patients with auto-HSCT, 3 year overall survival (OS) and disease-free survival (EFS) were 76.5% and 60.9%, respectively. (3)5/4110 patients with allo-HSCT, 3 year EFS and OS of allo-HSCT were 61.7% and 58.9%, respectively. (3)4/365 patients with CR1 status before auto-HSCT, 3 year OS and EFS were 87.3% and 68.7%, respectively. 20/56 patients with non-CR1 status before auto-HSCT, 3 year OS and EFS were 60.6% and 40.2%, respectively. The OS and EFS 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 EFS were 62.8% and 60.8%, 20/56 cases treated with auto-HSCT, the 3 year OS and EFS were 47.8% and 36.9%. The OS and EFS 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. Allo-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/MTX/ARA-C FOLLOWED BY ASCT AS FIRST-LINE THERAPY IN MANTLE CELL LYMPHOMA PATIENTS PROLONGS PROGRESSION FREE SURVIVAL TO MORE THAN 9 YEARS.**

**SINGLE CELL 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 with R-HyperCVAD. 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: 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 (90.0%) 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 22.6 (95%CI:21.2-23.9) months (11.4 years) for the whole group, 11.4 (47.3-180.7) months (9.4 years) for the transplanted patients vs 21.1 (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 lymphoid malignancies. 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 identified by PET/CT 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 suggestive 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 63 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:** The acquisition of additional chromosomal abnormalities are generally accompanied by the emergence of therapeutic resistance and eventually lead to poor treatment outcomes. However, the actual clinical impact of karyotypic evolution on prognosis differs depending on the type of hematologic malignancy. Although several prognostic indexes, including the International Prognostic Index (IPI), revised IPI (R-IPI), National Comprehensive Cancer Network (NCCN)-IPI, and Kyoto Prognostic Index (KPI) which we have developed (Kobayashi T. Blood Cancer J 2016), have the determinants for prognosis, little is known concerning the prognostic impact of karyotypic evolution in diffuse large B cell lymphoma (DLBCL), the most prevalent subtype of non-Hodgkin lymphoma.

**Aims:** We in this study investigated the clinical impact of karyotypic evolution on the treatment outcome of DLBCL.

**Methods:** We retrospectively reviewed 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-IPI, NCCN-IPI, and KPI. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

**Figure 1.**

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.
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 accompanies 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.

E961

EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1-MEDIATED OVEREXPRESSION OF MYC AND BCL2 CAN PREDICT POOR PROGNOSIS IN PATIENTS WITH EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

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Background: Recently double-hit lymphoma or double protein expressor lymphoma has been identified as a distinct group of diffuse large B cell lymphoma with poor prognosis. However, the expression status, clinical and prognostic effect of combined overexpression of MYC and BCL2 in extranodal NK/T-cell lymphoma, nasal type (ENKTL) are not known.

Aims: This study aims to explore the clinical and prognostic effect of combined overexpression of MYC and BCL2 in ENKTL.

Methods: Paraffin-embedded lymphoma samples from 53 patients with newly diagnosed ENKTL were studied using immunohistochemistry for MYC and BCL2, and fluorescent in situ hybridization (FISH) for MYC and BCL2 were done on 5 tissue sections with highest percentages of both MYC and BCL2 positive lymphoma cells.

Results: The median percentage of MYC-positive lymphoma cells and BCL2-positive lymphoma cells were 20% (range, 5% >45%) and 70% (10% >95%), respectively. Using median scores as cutoffs, we assigned each patient an IHC double-hit score (DHS) that ranged from 0 to 2. Using this DHS, 15 patients (28.3%) had a DHS of 0, 24 patients (45.3%) had a DHS of 1, and the remaining 14 patients (26.4%) had a DHS of 2. FISH analysis was performed on 5 tissue sections with DHS of 2, and none of them had MYC or BCL2 rearrangement. The DHS was not associated with patients’ age, gender, disease stage, LDH level, B symptoms, performance status, or local tumor invasiveness. However,
patients with tumor localized in extranasal sites seemed to have higher expression of BCL2 and higher DHS 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 who could be divided into three significantly different risk groups for PFS and OS (3-year PFS rate for DHS of 0, 1, and 2 was 60%, 41%, and 21%, respectively, P=0.008; 3-year OS rate for DHS of 0, 1, and 2 was 79%, 49%, and 33%, respectively, P=0.015). In multivariate survival analysis, it was found that DHS was an independent prognostic factor for both PFS and OS (P=0.006 and 0.011, respectively).

Summary/Conclusions: Our study demonstrated that DHS can help identify patients with newly diagnosed ENKTL who are at a high risk for a poor clinical outcome, which needs to be validated in prospective clinical trials with patients treated uniformly.

E962

SOLUBLE INTERLEUKIN-2 RECEPTOR AS A PREDICTIVE MARKER FOR SPONTANEOUS REGRESSION OF OTHER IATROGENIC IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS: A RETROSPECTIVE STUDY


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Background: Patients treated with immunosuppressive drugs (ISD) for autoimmune diseases are at an increased risk of developing other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OI-LPD). Some patients with OI-LPD shows spontaneous regression after withdrawal of ISD, but some require chemotherapy. The factors that are associated with spontaneous regression and outcomes of chemotherapy remain uncertain.

Aims: The aims of our retrospective study are to assess the clinical factors that predict spontaneous regression of lymphoma after ISD withdrawal in patients with OI-LPD and to evaluate the outcomes of patients who underwent chemotherapy without spontaneous regression.

Methods: We collected data from all patients with autoimmune disease who were pathologically diagnosed with OI-LPD between January 2002 to October 2016 at Yokohama City University Hospital, and Yokohama City University Medical Center.

Results: The patients included 12 males and 28 females, with a median age at diagnosis of 65 years (range 30-81). Methotrexate (MTX) was administered to all patients at any point of the clinical course before OI-LPD. The median time from diagnosis of autoimmune disease to OI-LPD development, and the median duration of MTX administration were 12 months (range 1-168) and 89 months (range 4-297), respectively. The histological findings of OI-LPD were diffuse large B-cell lymphoma (DLBCL) in 26 patients, follicular lymphoma in 4, and LPD in 4. EBER in 1, MALT in 2, peripheral T cell lymphoma, not otherwise specified in 3.

Table 1.

<table>
<thead>
<tr>
<th>Characteristics of Patients</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of patients (years)</td>
<td>18-60</td>
<td>80%</td>
</tr>
<tr>
<td>Stage</td>
<td>Early</td>
<td>57%</td>
</tr>
<tr>
<td>Performance status</td>
<td>good</td>
<td>77%</td>
</tr>
<tr>
<td>Intensity of DLBCL on biopsy</td>
<td>High</td>
<td>6%</td>
</tr>
<tr>
<td>Tumor cell expressing PD-1</td>
<td>None</td>
<td>40%</td>
</tr>
<tr>
<td>Presence of extracellular PD-1 in cells</td>
<td>None</td>
<td>40%</td>
</tr>
<tr>
<td>Intensity of PD-L1 in tumor</td>
<td>None</td>
<td>40%</td>
</tr>
<tr>
<td>Survival of patients</td>
<td>1-5</td>
<td>100%</td>
</tr>
</tbody>
</table>

Conclusions: Our study revealed that an sIL-2R level of ≥2,400 U/mL was significantly associated with spontaneous regression in patients with OI-LPD. Because CR rates with chemotherapy in patients without spontaneous regression are low, evaluation of sIL-2R in patients with OI-LPD may be useful for an early withdrawal of ISD, resulting in a higher chance of spontaneous regression.

E963

PROGRAMMED DEATH-1 PROTEIN EXPRESSION AND ITS RELATION WITH HISTOLOGICAL AND CLINICAL VARIABLES IN MYCOSIS FUNGOIDES

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Background: Mycosis fungoides (MF) is a T-cell malignancy with affinity for the skin. In early stages, treatment directed to the skin can induce long-lasting remissions. However, advanced stages are characterized by short-duration remissions and progressive disease. The programmed death cell surface protein-1 (PD-1) is expressed on activated T cells. Interactions between PD-1 and its ligands control the induction and maintenance of peripheral T-cell tolerance during the normal immune response. These interactions may also play a role in the immune evasion of tumors in which PD-1 ligand is overexpressed.

Aims: To described histologic characteristics and the proportion and intensity of PD1 expression by tumor cells, as well as the presence of PD1 positive lymphocytes in the epidermis in patients with MF. To identify histologic variables that might have an impact in clinical outcome.

Methods: Histologic 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-
trate, epidermotropism, cellular atypia, tumor density, presence of folliculotro-
pism and phenotypic alterations) and the proportion and intensity of PD1 expres-
tion by tumor cells, the presence of PD-1 positive lymphocytes in the epidermis. Likewise, a Pearson correlation analysis was performed between the degree of atypia and the ratio of PD-1 expression, PD-1 intensity, and loss of CD7 expression in tumor cells. Statistical analysis was performed using the IBM SPSS Statistics version 21.0.

Results: The median follow-up was 125 months (range 6-450 months). Char-
acteristics of patients are in Table 1. The overall survival (OS) at 10 years was 81%. OS in the early stages was 85% vs.64% in advanced stages (p<0.05). The OS for patients <60 years was 85%, and 75% for patients ≥60 years (p=0.05). Regarding histologic findings, the degree of atypia was the only variable that had an impact in OS. (see Figure 1) The presence of atypia grade 1 had an OS of 88%, grade 2 of 75%, and grade+3 of 50% (p<0.05). We performed a correlation analysis between degree of atypia and the ratio of PD-1 expres-
sion, PD1 intensity, and loss of CD7 expression. A positive correlation was detected, however it was weak (r<0.5).

Summary/Conclusions: MF tumoral cells express PD-1 protein in a high pro-
portion 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. E964

CIRCULATING MICRORNAS 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 treatment 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, prog-
osis 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 microRN-
As 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 (C5). In the case of an 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 could 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 R-
CHOP 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, biomarker of a disease pro-
gression, 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-19b and miR-19a. 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.

Summary/Conclusions: miR-197, miR-20a, miR-451, miR-122, miR-19a, miR-
19b, let-7e and miR-21 have been selected in this study and are currently quan-
tified in the plasma of the entire biobank. Since then, 19 patients have been 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 out-
come 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 includ-
ed. 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 radio-
therapy (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%, respec-
tively. 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], 10.522; 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, 8.920; 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.
Methods: Fourteen (4 female, 10 male) consecutive frail elderly patients (median age: 79 years; range 68-86 years) with a-B-NHL (11 DLBCL, 1 Burkitt NHL, 1 Burkitt-like NHL and 1 Mantle cell lymphoma) were enrolled in a phase II study with bendamustine 70mg/m² i.v. on days 1 and 2, rituximab 375mg/m² i.v. on day 1 and oral dexamethasone 20mg total dose on days 1-4 for four cycles. Frailty criteria were age >58 years, or age >70 years associated with 3 or more comorbidities or at least one grade 3-4 adverse event according to the cumulative illness rating scale (CIRS), as well as not self-sufficient or the presence of geriatric syndromes.

Results: Patients who showed complete (CR) or partial response (PR) after the fourth induction cycle of RD-BENDA started a consolidation course with four weekly doses of rituximab (375mg/m² i.v.) followed, in the case of persistence of CR or PR, by a maintenance treatment with monthly courses of lenalidomide (10mg/m², days 1-21). All patients performed G-CSF prophylaxis to avoid febrile neutropenia. Patients with progressive disease after RD-BENDA started maintenance therapy with monthly courses of full dose lenalidomide. PFS and OS were performed for the assessment of the response and overall survival after RD-BENDA induction course and after rituximab consolidation. After a median follow-up of 6 months (range 2-18), the overall response rate was 81%, with CR and PR of partial response rates of 63 (n=7) and 21% (n=2) respectively. Two patients died due to multiple organ failure and disease progression after 1 and 8 months from diagnosis, respectively. In our frail old, elderly patient cohort, the sequential treatment strategy was well-tolerated. After RD-BENDA cycles, grade II infectious disease was observed in 2/11 patients (18%) and DNA-CMV reactivation was detected in 2 other 2 additional patients (18%). However, 2 out of five patients who started maintenance lenalidomide discontinuation therapy experienced renal and hematological grade 3 toxicity. At the time of analysis, the estimated median 18-month progression free survival (PFS) and overall survival (OS) were 75 and 66%, respectively.

Summary/Conclusions: Our preliminary data show that sequential treatment with RD-BENDA followed by four weekly doses of rituximab and finally by lenalidomide maintenance is a feasible and safe therapy option in frail elderly a-B-NHL patients, but needs to be assessed in a larger subsequent trial.

E968

CLINICAL RELEVANCE OF SARCOPENIA IN DIFFUSE LARGE B-CELL LYMPHOMA - TWO ARE BETTER THAN ONE

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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 with standard front-line R-CHOP therapy. Furthermore, the synergistic role of L3- and PM-SMIs as prognostic markers was also investigated.

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 5-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.

E969

INTENSIFIED TREATMENT REGIMENS IMPROVE EVENT-FREE AND OVERALL SURVIVAL IN YOUNGER NEWLY DIAGNOSED HIGH-RISK PATIENTS WITH B-LARGE CELL LYMPHOMA: A REPORT FROM THE OBSERVATIONAL STUDY OF KROHEM


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Background: Standard therapy for newly diagnosed B-large cell lymphoma (B-LCL) is R-CHOP. Patients with high-risk disease have unsatisfactory outcomes. Non-randomized trials have suggested that intensified regimens, such as R-CHOEP14 and DA-R-EPOCH, improve treatment results in younger patients.

Aims: We performed this analysis to compare response rates, event-free (EFS) and overall survival (OS) rates of newly diagnosed patients with high-risk disease treated with R-CHOEP21 and more intensive regimens (R-CHOEP14 and DA-R-EPOCH).

Methods: Outcomes of B-LCL patients younger than 60 with aaIPI ≥2 treated at two different centres with R-CHOEP14 and DA-R-EPOCH were collected retrospectively from patient files and compared to outcomes of patients with same characteristics treated with R-CHOEP21 from the registry of KroHem, the Croatian Cooperative Group for Hematologic Diseases. All three regimens were administered according to standard guidelines for 6-8 cycles. Patients in PR or with initial bulky disease were irradiated after the end of systemic treatment. Twelve patients treated with DA-R-EPOCH were autografted in 1st remission.

Results: 54 patients were treated with R-CHOEP21, 40 with R-CHOEP14 and 22 with DA-R-EPOCH. R-CHOEP14 and DA-R-EPOCH treated patients did not differ in response rates, EFS and OS and were grouped together for further analysis. R-CHOEP treated patients had less frequently bulky disease (25% vs 49%, P=0.07) than more intensively treated patients; there was no difference in age, gender, stage, elevated LDH or PS ≥2. Patients receiving R-CHOEP had similar response rates as those receiving more intensive regimens (80% vs 85%, P=0.455), but inferior EFS (HR 2.12, 95% CI [1.04-4.2], P=0.028) and OS (HR 2.15, 95% CI [1.07-4.3], P=0.034) (Figure 1). 5-year EFS rates were 48% and 78% and 5-year OS rates 50% and 80% for R-CHOEP21- and more intensively treated patients, respectively. Differences in outcomes between R-CHOEP and intensified regimens remained significant in a multivariate Cox regression model adjusted for age, gender and presence of bulky disease (HR 2.45, 95% CI [1.11-5.4], P=0.026 for OS and HR 2.46, 95% CI [1.16-5.24], P=0.019 for EFS).

Figure 1.
SUBSTITUTING DOXORUBICIN WITH ETOPOSIDE IN R-CHOP RESULTS IN INCREASED DISEASE CONTROL, BUT AT THE COST OF INCREASED CARDIAC TOXICITY IN ELDERLY PATIENTS WITH LARGE B-CELL LYMPHOMA: SERBIAN EXPERIENCE

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Aims: The aim of this study was to evaluate the impact of the substitution of doxorubicin in the R-CHOP regimen with etoposide on the efficacy and toxicity of the new regimen in elderly patients with large B-cell lymphoma (LBCL) who were treated with this regimen in the Clinical Center of Serbia and the University of Belgrade.

Methods: From 2011 to 2016, 171 patients with LBCL aged 70 years or older were treated with the R-CHOP regimen. The substitution of doxorubicin with etoposide was evaluated in terms of disease control and side effects.

Results: The overall response rate was 90.5%, with complete response in 47.3% and partial response in 43.2%. The median progression-free survival (PFS) was 15 months, and the median overall survival (OS) was 30 months. The rate of grade 3-4 toxicity was high, with cardiac toxicity in 12.9% of cases, neutropenia in 25%, and neuropathy in 12%.

Summary/Conclusions: The substitution of doxorubicin with etoposide in the R-CHOP regimen resulted in increased disease control but at the cost of increased cardiac toxicity. This highlights the importance of considering cardiac toxicity in the treatment of elderly patients with LBCL.
E972

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER: 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 (8.9%) 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 2 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 III/IV in 73% of the patients (38). Among the 52, 45 received treatment (86.5%), 37 with immunochemotherapy (82.2%) and 8 with Rituximab (17.9%). 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 reactivation 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 patients seen in routine clinical care.

Methods: In this retrospective study, adult patients ≥18 years old with newly diagnosed DLBCL who had received a first-line therapy (1LT) for 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).

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.
### Bleeding disorders (congenital and acquired)

**E974**

**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 (Eloctate) 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, ELOCTATE (Factor VII with Fc Fusion Protein) on episodic basis after clinical bleed. In next 4 months they received prophylactic ELOCTATE, given intravenously at doses of 20 unit/kg body weight on twice weekly schedule. Annual bleeding rates, school absenteeism, emergency visits and other 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 0.84 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 VII 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.

### E976

**RETROSPECTIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF A-HYPO-FIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS**

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**Background:** Afibrinogenemia (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.

**Facts:** 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 hemorrhages. 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, 1concomitant aortic and inferior vena cava thrombosis occurred: 3 events during FC therapy, 1 during perioperative period. 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 patients 2 surgeries were performed after FC therapy, 3 in HF patients 2 surgeries were performed after perioperative treatment with LMWH. No visceral 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 hemorrhages, 1 DIC and 4 complicated pregnancies were recorded. FC was administered at delivery and LMWH during peripuerium, 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.

### E975

**NOVEL MUTATIONS IN THAI CHILDREN WITH CONGENITAL FACTOR VII DEFICIENCY**

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**Background:** Congenital factor VII (FVII) deficiency is a rare autosomal recessive coagulation disorder resulted from mutations in the FVII gene (F7). The disease severity is not correlated with FVII levels but might be determined by molecular defects in F7.

**Aims:** To delineate the phenotypic and genotypic characteristics of patients with congenital FVII deficiency.

**Methods:** We described demographic data, clinical manifestations, and outcome of patients with congenital FVII deficiency. F7 mutation analysis was performed by PCR-direct sequencing.

**Results:** Of the ten patients diagnosed with FVII 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 intracerebral hemorrhage within the first month of life, two presented with gastrointestinal bleeding and one presented with hemorrhaxis. 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 were novel (c.1192G>T (p.R391L), c.1318G>C (p.G437R), c.1192G>A (p.R391L), c.1318G>C (p.G437R), c.1192G>A (p.R391L), c.1318G>C (p.G437R), and c.1256C>T (p.T419M)) and associated with major bleeding especially during infancy.

**Summary/Conclusions:** This study reported Thai children with congenital FVII deficiency presented with life-threatening bleeding especially in the first year of life. Pathogenic including newly identified variants in the F7 gene were detected in all cases. Genetic counseling can be appropriately provided to reduce the risk of disease recurrence in the families at risk.

### E977

**RETROSPECTIVE REVIEW OF FOUR YEARS 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 pre for 2 days and 40 U/kg post for 2 days is safe and effective as the standard therapeutic dose of 40-60 U/kg preoperative and 20-40 U/kg post for 4 days in patients with vWD undergoing scheduled surgical procedures and for 4 days (one dose pre-op and one post-op) for one off centre due to the abbreviated surgical procedures.

**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 D0-3 for minor and major surgery. The definition of bleeding risk was based on NHLBI guidelines.

**Results:** Eighteen (20%) were males and 72 (80%) were females. Type I vWD constituted 94.4% (85/90). 84.4% Caucasian patients, 8.8% African American, 1.1% South Asian. Twenty-nine (32.2%) procedures were categorized as major surgery, 62 (66.6%) were minor surgery and 2 (2%) uncomplicated dental procedures. Eighty-four (93.3%) achieved excellent hemostatic efficacy defined as clinical hemostasis within normal limits. Six (6.6%) surgeries had good hemostatic efficacy defined as slight oozing. Five (6%) patients required blood transfusions.
(1-3 units packed red blood cells). 100% excellent-good outcomes similar to longer factor replacement schedules were noted. No deaths or thromboembolic events were noted. One patient required re-admission for post-op hematoma following gynecologic procedure. Thirty-four (37.7%) patients were discharged either same day or after overnight observation. Von Willebrand factor (VWF) is necessary to form a platelet plug by recruiting Factor VIII and platelets to damaged vessel walls. We believe that four days is sufficient for the formation a stable platelet plug and further downstream hemostasis is presumably VWF-independent. Here, we demonstrate safety and efficacy of a two to four day regimen of Humate-P, with only one person requiring re-admission for a post-operative hematoma. Current guidelines for surgical prophylaxis of VWD are based on expert opinion and lack level 1 evidence. Prolonged exposure may place patients at risk for unnecessary side effects, including thromboembolism and protracted hospitalization, and also causes financial toxicity. One unit of Humate-P costs 1.2 USD, which translates to enormous cost savings. Cumulatively, we were able to save ~1.5 million USD in these 90 surgical events using our abbreviated schedule.

Summary/Conclusions: Perioperative dose of 40U/Kg for 2 days (one dose pre-op and one post op) for extensive dental procedures and for 4 days (one dose pre-op and for 3 days post-op) for minor and major surgical procedures are associated with excellent hematologic outcomes and significant cost savings. However, this is single institutional data with limited number of patients and there remains a need for further studies to better define the exact dosing and duration of surgical prophylaxis.

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 antide. 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.

Aims: 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 9/16 (56.2%) non-bleeders with an INR between 5-8. Additionally, the recommended dose and route of administration of Vitamin K 1-5mg PO was followed only in 7/16 (44%) of non-bleeders with INR >8.

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. Aims: 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).

Six (38%) patients have 2 mutant alleles and three mutations were recurrently identified. The most frequent mutation detected in this study was Cys389Gly detected in 37% (11/30) patients, validating the data of our previous patient cohort.

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.
**Bone marrow failure syndromes incl. PNH - Clinical**

**E980**

Abstract withdrawn.

**E981**

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-PerCP, CD55-PE-Cy5.5, CD14-PerCy7, CD64-APC, CD45-APC H7. We assessed the utility of single tube 5 color combination of FLAER-AF488, CD15-PE, CD15-PerCP, CD55-PE-Cy5.5, 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 range and calculating the coefficeint of variance (CV). Correlation of PNH clone size obtained from CD24/CD14 and CD157 was assessed by analysing a total range and calculating the coefficeint of variance (CV). Correlation of PNH clone size obtained from CD24/CD14 and CD157 was assessed by analysing a total range and calculating the coefficeint of variance (CV). Correlation of PNH clone size obtained from CD24/CD14 and CD157 was assessed by analysing a total range and calculating the coefficeint of variance (CV).

Results: CD157 was sensitive at the level of 10^-2 and better. Frequency of cells with PNH phenotype in normal samples were found to be <0.002%. The CVs of intra-assay precision analysis ranged from 0.92% to 3.24% for granulocytes and 1.92% to 5.36% for monocytes. The PNH clone size 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.

**E982**

IMMUNOPHENOTYPIC DYSPLASTIC FEATURES IN PATIENTS WITH APLASTIC ANEMIA

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Background: Multicolor flow cytometry (MFC) of bone marrow (BM) is a promising additional approach to the diagnosis of myelodysplastic syndromes (MDS). Aplastic anemia (AA) as MDS characterizes by cytopenias and dysplastic features in BM by morphology are absent. It is well known that up to 15% of AA transformed in MDS over time. It is possible to suggest that in some cases of AA immunophenotypic abnormalities can also be identified.

Aims: To study and compare the presence of dysplastic features by MFC in AA and MDS without excess of blasts.

Methods: The study included 14 patients with AA (8m, 6f, median age 33), 28 patients with MDS de novo without excess of blasts by morphology (13m, 15f, median age 59). MDS group included 3 patients with 5q-syndrome, 4 - RCUD, 3 - RARS, 18 - RCMD. 20 patients with cytopenias constituted the control group (4m, 16f, median age 42) due to B-12 deficiency anemia, iron-deficiency anemia, Fanconi anemia, hemolytic anemia, β-thalassemia, ITP, hepatitis C, multiple myeloma, Burkitt’s lymphoma. BM of 33 healthy donors was used as the reference values. MFC was performed according to International Leukemias and Myelodysplasias Study Group. The reference values were adopted for the reference values. MFC was performed according to International Leukemia and Myelodysplasias Study Group. The reference values were adopted for the reference values. MFC was performed according to International Leukemia and Myelodysplasias Study Group. The reference values were adopted for the reference values. MFC was performed according to International Leukemias and Myelodysplasias Study Group. The reference values were adopted for the reference values.

Results: Among MDS patients without excess of blasts assessment “B” and “C” scores were obtained in 78.6% (sensitivity). Increased proportion of CD34+ myeloblasts was in 35.7% of cases, increased CD56 and CD7 - in 42.9%. The most common abnormalities were: increased CD66 (53.8%), abnormal patterns (39.3%), low granularity (35.7%) in granulocytes; increased expression (21.4%) and abnormal patterns (28.6%) in monocytes. 64.3% (n=9) patients with AA (100%) were assessed as “A”, 21.4% (n=3) as “B” and 14.3% (n=2) as “C” with high concordance of “B” and “C” assessment. Increased expression of CD117 and expression of CD56 on CD34+ cells were seen. AA patients with “B” and “C” showed increased expression of CD66, CD64 and decrease CD10 expression on granulocytes. Abnormal patterns were less common than in MDS patients. The increased proportion and CD64 expression in monocytes were more frequent than in MDS patients. All patients not diagnosed with AA, MDS were assessed as “A” (specificity 100%). But some MFC abnormalities were found in them: abnormal expression of CD34 (35%) and CD45 (30%) on CD34+ myeloblasts, and increased CD64 expression (20%) on granulocytes (pic.).

Summary/Conclusions: Flow cytometry MDS study with Ogata/Wells scale has a high sensitivity and specificity. Immunophenotypic abnormalities characterizing dysplastic features can also be found in AA patients up to 35% of cases. Increased expression of CD56 on CD34+ myeloblasts, granulocytes and monocytes is commonly found in AA patients. Perhaps the appearance of MFC dysplastic features foreshadows the MDS-transformation of AA, but requires further prospective studies.

**E983**

SURGICAL MANAGEMENT OF PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) – DATA FROM THE SPANISH PNH REGISTRY

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Background: Multicolor flow cytometry of bone marrow (MFC) is a promising additional approach to the diagnosis of myelodysplastic syndromes (MDS). Aplastic anemia (AA) as MDS characterizes by cytopenias and dysplastic features in BM by morphology are absent. It is well known that up to 15% of AA transformed in MDS over time. It is possible to suggest that in some cases of AA immunophenotypic abnormalities can also be identified.

Aims: To study and compare the presence of dysplastic features by MFC in AA and MDS without excess of blasts.

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 splenoportal 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 7; 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 hemorrhagic 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 7 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).

Table 1.

<table>
<thead>
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<th>Table 1.</th>
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<td><strong>Summary/Conclusions:</strong> 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 in those with a previous history of surgery-related hemolysis.</td>
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<td><strong>E984</strong></td>
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</table>

**EFFICACY OF ECULIZUMAB IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS WITH OR WITHOUT APLASTIC ANEMIA:**


**Methods:** The prospective study of Korean PNH cohort includes 46 total patients, 12 (26%) were classified as having PNH/AA and 34 with classic PNH. 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 outcomes between the two groups. Treatment with eculizumab induced a rapid inhibition of hemolysis. At the time of 6 month 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 to 3.5 months after 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 PAROXYSMAL 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 GPI-AP 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 2009 in Saint-Petebsurg, Russian Federation.

Methods: A PNH clone has been researched in 234 patients since August 2009 in Saint-Petebsurg, Russian Federation.

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 who 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), meyoldysplasia (MDS), unexplained cytopenia and thrombosis. The search for the PNH clone by FC is based on the analysis of the following monoclonal antibodies: FLAER and CD59 on neutrophils, CD55 and CD45 on monocytes and CD59 with gating on Glycophrin A for red blood cells. We judged that the patient has a PNH clone when the deficiency is >50% on at least two markers highlighted on two different lines. FC surveillance is provided in the absence of a deficit or in case of the recovery 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 recovery of TNFα and IL-4. This pattern, taking into consideration our earlier obtained data, may be the evidence of the role of NK-T cells in regulation of balance Th1:Th2 and produced by them cytokines.

Table 1. NK-T cell level (%) in patients with AA in remission according to subgroups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Remission duration (months)</th>
<th>Residual hemoglobinaemia (%)</th>
<th>Residual thrombosis (%)</th>
<th>IST-free period (months)</th>
<th>Size of PNH-clone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary AA</td>
<td>1</td>
<td>0.2%</td>
<td>0.3%</td>
<td>-</td>
<td>0.1-1%</td>
</tr>
<tr>
<td>Remission</td>
<td>2</td>
<td>0.4%</td>
<td>0.5%</td>
<td>1-2</td>
<td>1-2%</td>
</tr>
<tr>
<td>Complete (CR)</td>
<td>2</td>
<td>0.6%</td>
<td>0.7%</td>
<td>1-2</td>
<td>1-2%</td>
</tr>
<tr>
<td>Duration of IST-free</td>
<td>2</td>
<td>0.8%</td>
<td>0.9%</td>
<td>1-2</td>
<td>1-2%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The role of NK-T cells in development of aplasia of AA is now 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) completeness of remission (CR); 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 trend in their dynamics in all assigned subgroups, except for NK-T cells. In primary AA patients the level of NKT cells in PB and BM exceeded normal level 1.8- and 2.2-fold, respectively. In patients with remission ≥36 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, NKT 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 NKT cells in PB and BM. It is known that PNH-clone presence is a favourable factor for treatment response. Therefore, in order to study the association 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.

E987
A NOVEL DUAL-REAGENT SINGLE TUBE FLOW CYTOMETRIC ASSAY TO SCREEN PAROXYSMAL 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 PNH-related anti-gens on at least two cell lineages. Alexea fluor 488 conjugated fluorescent Aerolysin (FLAER-AF488) has become a mandatory component in FCN based PNH assays.

Aims: We have analyzed the feasibility of a novel dual-reagent assay for a single tube, single tube, direct coombs test for detecting PNH.

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/APC. Simultaneously, the routine two tube flow cytometry assay (established sensitivity of 0.1%) for detection of PNH for TNFα and IL-4 was performed (21 cases). Inclusion of granulocytes and FLAER/CD33/CD14 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 granulocyte and monocyte PNH positivity and the respective clone sizes determined 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. Thus, the presented strategy was more effective for reporting PNH-clone in granulocytes and monocytes by both strategies, indicating complete concordance at a sensitivity of 0.2% (Chi Square p<0.000). Of the PNH positive cases, the mean PNH clone sizes among the granulocytes by dual-reagent and conven-
tional methods were 3.79% (range, 0.2-18.2) and 3.60% (range, 0.1-28.8), respectively. The mean PNH clone sizes among the monocytes by dual-reagent and conventional methods were 7.30% (range 0.2-29.4) and 7.32% (range 0.1-28.8), respectively. There was no significant difference in the granulocyte and monocyte PNH clone sizes determined by both the methodologies (p>0.000). There were significant correlations between the granulocyte PNH clone sizes (Pearson’s r=0.993, p=0.000) and the monocyte PNH clone sizes (Pearson’s r=0.991, 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%.

**E988**

**TREATMENT OF REFRACTORY APLASTIC ANEMIA WITH ELTROMBOPIAG: EXPERIENCE OF A CENTER**

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1Clinical Hematology, 2Clinical Pathology, São João Hospital Centre, Porto, Portugal

**Background:** Ertrombopag, 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) refractory to immunosuppressive therapy.

**Aims:** In this report, we evaluated response to ertrombopag in patients with refractory severe hematologic response of one or more hematopoietic lineage, independence of side effects occurred.

**Methods:** Retrospective analysis of six patients with a diagnosis of aplastic anemia and thrombocytopenia (platelet count ≤30,000/μl), refractory to immunosuppressive therapy and ineligible for allotransplant, treated with ertrombopag. Patients characteristics, response, clinical evolution and adverse effects were evaluated.

**Results:** Four patients were female and median age at diagnosis was 66 years (36-76). Previous treatments included horse antithymocyte globulin (1), cyclosporine (4), intravenous immunoglobulin (1), corticosteroids (4) and danazol (1). Treatment with ertrombopag was associated to cyclosporine in four patients; two cases had chronic renal failure and consequent contraindication to cyclosporine. The median duration of treatment with ertrombopag at the time of this analysis was 7 months (3-12). At 3 months, all patients had platelet counts >30,000/μl (median increase, 16,500/μl). Five patients improved hemoglobin levels (median increase, 2.2g/dL); 3 of them were previously dependent on red cell transfusions, and no longer needed transfusions. Four patients increased neutrophil counts (median increase, 1110/μl). All but one patient received a maximum dose of 150mg per day. Only one patient needed temporary discontinuation due to hepatic abnormalities, that were rapidly resolved. One other patient had mild elevation of liver enzyme levels. No other relevant side effects occurred.

**Summary/Conclusions:** Treatment with ertrombopag was associated with hematologic response of one or more hematopoietic lineage, independence of blood transfusions and improved quality of life of patients with refractory severe AA. Except for infrequent and reversible hepatic abnormalities, tolerability was excellent. Thus, ertrombopag might be used in situations where other measures have failed in patients who have no indication for allogeneic stem cell transplant. A caution, however, should be taken on the risk for clonal evolution that might be further standardized to achieve a sensitivity of 0.01%.

**E989**

**DECREASED EXPRESSION OF ADHESION MOLECULES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS OF PATIENTS TREATED WITH IBRUTINIB**

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1Department of Cellular Biotechnologies and Hematology, 2Department of Molecular Medicine, Hematology, Sapienza University, Rome, Italy

**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 treatment on the relationship between the microenvironment, that promotes cell survival and proliferation, and the leukemic 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 CLL1114 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 FACSCanto II, BD) of CD11a, CD18, CD38, CD40, CD43, CD44, CD49d, CD62L, CD69, 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±5.88 vs 53.4±5.10 x 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±146; 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. 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); this antigen is utilized in CLL for the detection of minimal residual disease (MRD) 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±887; p<0.001), while we unexpectedly observed the up-modulation of CD16 (2244±2022 vs 3182±1877; p<0.0003); both antigens participate in the BTK signaling pathway. CD40, that interacts with the 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 increased (p<0.028 and p<0.021) down-modulated: both molecules have a role in the crosstalk between the leukemic cells and the microenvironment. No significant changes were detected in the expression of CD11a, CD18, CD44, CD60, CD86 and CD154.

**Summary/Conclusions:** Within an ancillary biologic study of the GIMEMA CLL1114 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.

**E990**

**CLL CELLS UNDERGO METABOLIC REPROGRAMMING AND UTILIZE FREE FATTY ACIDS AS THEIR PRIMARY ENERGY SOURCE**


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**Background:** We previously demonstrated that CLL cells undergo metabolic reprogramming and utilize fatty acids as their primary energy source. This mechanism contributes to the progressive resistance to ibrutinib treatment and supports the maintenance of myeloid-like signature of the leukemic cells.

**Aims:** We aimed to validate the role of free fatty acids (FFA) in the metabolic reprogramming of CLL cells, and to elucidate the proteomic changes associated with this process.

**Methods:** We carried out a proteomic analysis of CLL cells, under normal medium condition (control) and in the presence of 5mM palmitate (a saturated FFA) as a source of FFA. Protein expression was analyzed by Tandem Mass Tag Protein Profiling (TMT) and identified by LC-MS/MS on a Orbitrap. We performed a differential analysis to identify proteins significantly changed by FFA supplementation. In addition, we assessed the expression of selected genes associated with metabolic reprogramming by qRT-PCR.

**Results:** We observed a significant downregulation of proteins involved in mitochondrial ATP production (complexes I, IV, V andsuccinate dehydrogenase) and upregulation of proteins involved in FFA metabolism and utilization, such as peroxisomal fatty acid CoA ligase and mitochondrial acyl-CoA synthetase. Moreover, we identified a signature of proteins involved in the immune-stimulatory pathway, indicating a shift toward a more aggressive phenotype. We also observed a downregulation of the B-cell receptor (BCR) pathway components, consistent with the impact of ibrutinib treatment. Finally, we found a correlation between the proteomic changes and the expression of genes involved in metabolic reprogramming, supporting the role of FFA in the modulation of the metabolic phenotype.

**Summary/Conclusions:** Our findings provide insights into the metabolic reprogramming of CLL cells and support the role of FFA in the progressive resistance to ibrutinib treatment. These results may pave the way for the development of new therapeutic strategies targeting the metabolic pathways in CLL cells.
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).

Aims: To 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 immunoprecipitation (CHiP) 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 them and determined the concentration of cultured media-dissolved O2 (dO2) prior to and after adding FFA, assuming that if the cells oxidize the acid, dO2 levels will drop. Indeed, after 48 hours incubation with FFA dO2 levels were markedly reduced as compared with the dO2 levels in media of non-transfected cells. Indeed, dO2 levels in cultured CLL cells dropped to about 4% of the levels in media without FFA. Intriguingly, the levels of dO2 remained unchanged if CLL cells were incubated in the presence of FFA and ibrutinib. Similarly, the dO2 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. Similar to adipocytes CLL cells express LPL which mediates the uptake of lipid particles into the cells and catalyze the hydrolysis of triglycerides into free fatty acids. Indeed, freshly isolated CLL cells expressed LPL in the cytoplasm and in the cytoplasm of CLL cells. Furthermore, using small interfering RNA (siRNA) we knock-downed 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 confirmed the binding of STAT3 to the LPL promoter. Furthermore, transfection of CLL cells with 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.
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, PLCγ2, AKT and ERK, suggesting that downregulation of FcγR is the primary mechanism through which IL-4 regulates the BCR signaling capacity of 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 immunomodulatory processes.

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 purity 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 lymphoproliferative leukemia (B-CLL) patients (median: 67y). Healthy donors (HD) between the ages of 20 to 90 years subdivided into <50 years (median: 36yo) and >50 years (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 gender (p<0.01; <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; p<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 both significantly correlated with BCR and PD-1 expression (r=0.87). 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. CD4+ T cell 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 CD4+ T 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 contribute to a higher probability of progression, such as genetics, immunosenescence, and environment, cannot be easily identified and it is 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 certain 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 CD155 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, LA93, TIM3 and CTLA4. We also analyzed the expression of the transcription factors BACH2/PRDM1.

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 in % of CD8+ T cells (P=0.0186) 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+ T cells expressing PD1 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 different subclusters, the EM and CD45RA+ (TEMRA) CD8+ T cells expressed the highest percentages of CD244 and CD160. We did not observe changes in LA93, 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 Eomes and PD1 was 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 (EomeshiPD1hi) is predominant. These changes may contribute to the immune evasion that facilitates the progression and to the immunosuppressive scenario that dominates advanced CLL stages. 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 DICKENPONT AND 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 ibrutinib. 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 larger proliferative component.

**Aims:** The aim of this study was to investigate the effect of ibrutinib in vivo on the clonal expansion 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 µ chain was assessed by immunoblotting as a readout of slgM expression and function. Flow cytometric analysis was obtained from 7 patients (REC: H22/02/01).

**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 from other BCR-complex associated molecules. In the initial phase, the increased slgM expression and maturation, with no changes of 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.

**E998**

**ROLE OF THE COMBINATION MEK1/2 INHIBITOR BINIMETINIB AND AKT INHIBITOR MK2206 IN CLL**

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**Background:** Clinical trials of ibrutinib andidelalisib 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 MK2206 and idelalisib at doses varying from 1 to 40µM were tested on primary CLL cells. Secondly, binimetinib and MK2206 were tested as single agents and in combination at 20µM against primary CLL cells. Thirdly, binimetinib at 20µM combined with varying doses of idelalisib on primary CLL cells. The mechanisms underlying 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:** MK2206 is effective against CLL 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 alone, suggestive of synergy between the two drugs (Figure 1B). The analysis of binimetinib at 20μM with idelalisib failed to demonstrate any additive effects or suggestion of synergy between the two drugs (Figure 1C). Binimetinib treatment led to an increase in the activity of AKT and a decrease in ERK1/2 phosphorylation. MK2206 completely abrogated the activation of AKT and MCL-1 phosphorylation when combined with binimetinib (Figure 2A). Although we observed a reduction in AKT phosphorylation following idelalisib alone, it had no effect on the levels of AKT activity induced by binimetinib or the levels of phosphorylated MCL-1 protein. This result was irrespective of the dose of idelalisib used (Figure 2B). We explored the possibility that protein kinase C (PKC) may be involved in binimetinib-induced AKT phosphorylation. Using the pan-PKC inhibitor GF109203X (GFX), we demonstrated that inhibition of PKC significantly reduces binimetinib-induced phosphorylation of AKT with no effect on the activity of ERK1/2-MAPK (Figure 2C). These data suggest a role for PKC in the regulation of AKT activity in CLL cells.

Summary/Conclusions: The combination of binimetinib and MK2206 in vitro has been shown to be effective strategy to treat primary CLL cells. The western blot data reinforce that the increased activity observed in AKT activity in CLL cells following binimetinib treatment is independent of the idelalisib and totally abrogated by MK2206. This PIS kinase-independent regulation may be regulatively different and is likely to play a significant role. Dual 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-1α 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. We have previously shown that co-culture with stromal cells (SC) induces in CLL cells the activation of RhoA/RhoA kinase and Ras/ERK1-2 signaling, the upregulation of Akt, and an increased 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), HF-1α inhibitor BAY89-2243 (1 μM), and P13K 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 amount in whole cell extracts and in nuclear fraction, and HIF-1α phosphorylation were evaluated by Western Blot. RhoA kinase, Akt and HIF-1α activities were measured with specific immunoassay kits. CXCL12 was quantified by ELISA. Cell viability was determined by Annexin-V/propidium iodide immunostaining and flow cytometry analysis.

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 unveiling 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: 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 to 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, CYCLOPHOSHAMIDE AND OFATUMUMAB MAB

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Background: Chemomunotheraphy with fludarabine, cyclophosphamide and rituximab is the optimal front-line treatment for fit chronic lymphocytic leukemia (CLL) patients. IGHV mutations and FISH lesions are predictive marks 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 7PS3, NOTCH1, BIRC3 and SF3B1 mutations can predict the obtaining 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: flow 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.
17p was the consequence of an unbalanced translocation (n=167/240, 70%), abnormalities were found in the 195 patients. In the majority of cases, loss of the sole abnormality detected by K in 28/195 (14%) cases. A total of 240 17p+, 13 months and a median of 2 lines of treatment [1-10]. In 28/124 (23%) cases, years [33-88], 59% were Binet stage A, 28% B, 12% C.

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 survival between groups.

Methods: A total of 195 CLL patients harboring a 17p deletion were included in this study. All the K were reviewed by the members of the study group.

Summary/Conclusions: Among the high risk group of 17p- CLL, i(17q) confines a shorter OS than the other 17p abnormalities. In addition, the gain8q aggravates 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.

**E1001**

ISOCHROMOSOME 17q, UNBALANCED TRANSLocations AND 8q GAIN REPRESENT ADVERSE PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH 17p DELETION. A GFCH STUDY

**E1002**

THE MICROENVIRONMENT REGulates THE EXPRESSION OF MiR-21 AND TUMOR SUPPRESSOR GENES Pten, Pias3 and Pcdh4 THROUGH ZAP-70 AND THE BCR SIGNALING PATHWAY. A Laboratory for Cytogenetics, Chromosomal abnormalities are present in about 80% of CLL. Among them, the loss of the short arm of chromosome 17 (17p-), unbalanced translocations, deletions, gains and 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.

Background: Chromosomal abnormalities are present in about 80% of CLL. Among them, the loss of the short arm of chromosome 17 (17p-), unbalanced translocations, deletions, gains and 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.

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), MAPK (PD98059) and STAT3 (US-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 microenvironment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels of primary miR-21, miR-21, PTEN, PDDC4 and PIA3S 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 fused with Green fluorescent protein (GFP) or GFP only as a control we treated with Akt (LY294002), MAPK (PD98059) and STAT3 (US-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 microenvironment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels were measured using primary miR-21, miR-21, PTEN, PDDC4 and PIA3S 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 fused with Green fluorescent protein (GFP) or GFP only as a control we treated with Akt (LY294002), MAPK (PD98059) and STAT3 (US-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 microenvironment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels were measured using primary miR-21, miR-21, PTEN, PDDC4 and PIA3S were measured by QRT-PCR.

Summary/Conclusions: Among the high risk group of 17p- CLL, i(17q) confines a shorter OS than the other 17p abnormalities. In addition, the gain8q aggravates 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.
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 with reduced doses) or BR (bendamustine, rituximab); all harbored intact TP53 gene as assessed by FISH and the yeast functional analysis; 46/53 (87%) had unmutated IGHV. The next generation sequencing using MiSeq (Illumina) was done in 53 pre-therapy samples and 41 relapsed samples using three separate pan-els: ATM (exons 2-6; median coverage (MC) 6100), SF3B1/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 recommendations).

Results: In the pre-treatment analysis, we identified 23 patients with one disrupted gene and 7 patients with two disrupted genes; the rest of the cohort analyzed consisted of 15 patients (37%) with three or more disrupted genes, followed by 6 patients (11%) with two disrupted genes; the rest of the cohort (43%) did not observe any mutations. In the pre-therapy analysis, we identified 23 patients with one disrupted gene, 10 patients with two disrupted genes, and 10 patients with three or more disrupted genes.

In the pre-therapy analysis, we identified 23 patients with one disrupted gene, 10 patients with two disrupted genes, and 10 patients with three or more disrupted genes.

Summary/Conclusions: Our pilot analysis with limited number of samples does not indicate an adverse impact of studied mutations in rituximab-based regimens.

E1004 BCR SIGNALLING PROFICIENT CHRONIC LYMPHOCYTIC LEUKAEMIA B CELLS ARE PRONE TO RITUXIMAB MEDIATED ELIMINATION IN VIVO

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Background: Anti-CD20 monoclonal antibody rituximab (RTX) has improved clinical outcome of patients with CD20-positive B-cell malignancies, including chronic lymphocytic leukaemia (CLL). However, despite the fact that RTX has been clinically used for 20 years, the exact mechanism of its action remains largely unclear.

Aims: The aim of this study was to determine susceptibility of CLL cells’ subpopulations to RTX.

Methods: Peripheral blood samples from CLL patients (N=17) were obtained and analysed before (day 0) and 24 hours (day 1) after RTX administration (375mg/m², single agent).

Results: It was described that 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 CD55 on their surface. Furthermore, we showed that rituximab-sensitive cells had higher CD20 expression than CD20 resistant CLL cells (N=40; P=0.001). 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 CXCR4dimCD55high subpopulation after the first RTX dose (8.3% pre-RTX vs 2.1% post-RTX, P<0.0001). We further demonstrated that CXCR4 signaling proficiency of the CXCR4dimCD55high CLL subpopulation, since CD20 was proposed to play a role in BCR signalling. We observed that CXCR4dimCD55high CLL cells have higher immunoglobulin (IgM) expression (~2-fold, P<0.005) which was coupled with higher responsiveness to BCR crosslinking with anti-IgM (P=0.005). Moreover, CXCR4dimCD55high cells also have higher levels of CD19 (18, P=0.001), which is an important component of BCR complex that augments signal transduction. Furthermore, we demonstrated that CXCR4dimCD55high cells have higher phosphorylation of several proteins involved in PI3K/BCR/NFκB signalling pathway (P=0.05) compared to CXCR4dimCD55low cells obtained from the same patient. This led us to hypothesize that CXCR4 signaling proficiency 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 CXCR4dimCD55high CLL subpopulation is more sensitive to RTX compared to peripheral blood of CLL patients has the highest surface levels of CD20 and is therefore preferentially and effectively eliminated by RTX. These CLL cells likely represent the most “aggressive” subclone of CLL cells since they have relatively high proliferative and BCR signalling capacity.

Supported by: the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601); Czech Science Foundation (project No. 16-13334Y); the Ministry of Health of the Czech Republic, grant No. 16-29622A. All rights reserved. The European Union’s Horizon 2020 research and innovation programme under grant agreement No. 692298. This study reflects only the author’s view and the Research Executive Agency is not responsible for any use that may be made of the information it contains. M.H. is a recipient of a fellowship from the Italian Association for Medical Research (AIRC).
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 of c-Cbl in the development 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.

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 isolated from 37 CLL patients. Freshly isolated CLL cells incubated with increasing concentrations of nintedanib (0-24 µM) and MP07-66 (2,2-diethoxyethyl)[4-(4-hexylxoyl)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, SHP-1 and PPP2Ac expression and phosphorylation were evaluated by Western Blotting.

Results: We performed in vitro caspase 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 phosphorylated (at Ser591), 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 Ser591 phosphorylation of SHP-1 could be dephosphorylated by PP2A. In this scenario, the restoration of PP2A activity by a finkolimod analogue which 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 is triggered by specific small molecules caused stimulate each other's activity, 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.

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) (Doman ska et al., 2013) and binding to its ligand (CXCL12) 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 evaluated: 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. Cholesteryl 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 focusing on optimising liposome characteristics for binding and uptake by CLL and the delivery of cytotoxic payload.

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 using stealth-liposomes (Allen and Collins, 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 B-cells were activated with anti-CD45 and goat-anti-rabbit IgG antibodies, high-dose IL2 and TPO for 5 days. Percentage of cells retaining 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 patients’ T-cells with TPO led to a 12% increase in the number of CD4+ T-cells (from 7.5%±5.4 to 8.5%±6.4; p<0.05; n=8), whereas pro- liferation of healthy donor T-cells remained unaffected by TPO (11.5%±5.7 and 11.4%±5.7 of G0 in p=NS; n=6). Additionally, TPO stimulation resulted in a 24% increase of Treg levels in patient T-cells (from 2.1%±1.7 to 2.6%±1.7%; p<0.01; n=8). However, the Treg levels were not altered in healthy donor T- cells subjected to TPO (0.74%±0.7 and 0.74%±0.8; p=NS; n=5), which is similar to their proliferation response to this growth factor. To determine whether CLL B-cells could be the TPO source in this disease, TPO mRNA expression in the malignant cells was assessed, demonstrating a baseline ct value of 721±296, which significantly increased to 1033±342 (p<0.05; n=6) upon ODN activation.

Summary/Conclusions: In the current study, TPO is found to affect immune properties of CLL patient T-cells, inhibiting their proliferation and increasing Treg levels. These effects have been observed in patient T-cells only, which could be partly explained by higher levels of TPO-R expression revealed on patient T-cells compared to healthy donor T-cells. The elevated TPO mRNA expression in CLL B-cells could point to them as one of the possible sources of this growth factor in patient serum. Activation of TPO-R may represent a novel mechanism of T-cell inhibition in CLL.

E1009
TREATMENT WITH BCR INHIBITORS INCREASES ROR1 EXPRESSION IN CLL CELLS
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Background: Receptor Tyrosine Kinase-Like Orphan Receptor-1 (ROR1) expression on malignant B-cells is considered a promising target for therapy of CLL and other lymphoproliferative disorders. Recently published data suggest that combination of BCR inhibitor ibrutinib with ROR1 antibody cirtumumab can enhance treatment efficiency in CLL. Nevertheless, the variability in ROR1 expression in different stages of the disease using flow cytometry and qRT-PCR with focus on patients undergoing therapy; ii) analyze 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 with ibritinib before first therapy. ROR1 antigen changes in ROR1 expression we tested serial samples from 5 CLL patients (median follow up 76 months (66-131), median number of sampling points 12 (5-18)). For surface ROR1 protein analysis we used 8-colour flow cytometry (modified MRD protocol: CD45/CD3/CD19/CD5/CD81/CD79b/CD22/ROR1) in all samples. To quantify ROR1 mRNA expression changes within individual patients we performed qRT-PCR in serial CLL cells (>95% CD19+CD5+). CLL cells from samples in remission were separated immunomagnetically (Whole Blood Anti-ROR1 MicroBead Kit, Miltenyi Biotec).

Results: Using multicolour flow cytometry we confirmed ROR1 antigen/protein expression in CLL cells. We observed increased samples in patients treated with ibritinib, meanwhile 65,2% of patients with abnormal SPE exhibited at least one poor cytogenetic abnormalities such as del17p, del11q, del13q and trisomy 12 (assessed by standard cytotype, FISH analysis and QMPSF).

Summary/Conclusions: ROR1 antigen remains detectable on CLL cells during disease course even in complete remission. ROR1 mRNA levels are highly influenced by therapy administration especially in the case of treatment with Bcr inhibitors.

E1011
HSP70 EXPRESSION IS MODULATED BY ITS MASTER REGULATOR HSF1 VIA PI3K AND PI3KAKT/MTOR PATHWAYS IN CHRONIC LYMPHOCYTIC LEUKEMIA
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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. Very recently, we 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 and RAS/RAF/MEK/ERK). Aims: Since HSP70 is overexpressed in CLL neoplastic B cells and most of “HSF1-1-phosphorylating agents” belong to signalling pathways taking part from RAS, being the PI3K/AKT/mTOR and the RAS/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 subjects, we evaluated the activation/expression of key signalling proteins. Herein, we focused on HSP70, AKT-Ser473, mTOR-Ser244, GSK3α/β-Ser21/9, CDK2, CREB, Ser133, MEK1/2-Tyr202/204, ERK1/2-Tyr202/204, NFκB-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/β 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
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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 cells 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 (in), followed by a 5 h stimulation with PMA, ionomycin and Monensin (PIM), or with Anti-Tbet PE or anti-GATA-3 PE. Statistical analysis were carried out using the paired and unpaired two-tailed Student’s t-tests and confirmed with the non-parametric Wilcoxon signed-rank test.
Results: In CLL patients we observed a reduced production of IFN-γ 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-γ+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-γ-producing T cells resulted statistically significant 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-γ 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-γ 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 antitumor therapy in CLL.

E1013
LOW EXPRESSION OF CD25 IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Recently, it has been shown that CDK6-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 mutation 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. Paired-end sequencing was performed with NGS using 454 platform in 147 CLL patients. The frequency of mutations was 16.3% 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 assessing 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**


Aims: To analyze the presence of mutations of a panel of genes by NGS and 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 13q deletion and other prognostic markers associated with good prognosis.

Methods: Amplicon-based-NGS was performed using 454 platform in 147 CLL patients to evaluate the mutational status of 13 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-).

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, 23.8% 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 had 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 deletion (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 unmaturated 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.
E1016

ASSOCIATION OF CpG-STIMULATED KARYOTYPE WITHTIME-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.

<table>
<thead>
<tr>
<th>Continuous Characteristic</th>
<th>n</th>
<th>Number (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range) min, max</td>
<td>901</td>
<td>62 (24-91) 134 (0)</td>
</tr>
<tr>
<td>WBC, ALK/Cut, IGH (lgG monoclonal)</td>
<td>498</td>
<td>20.1 (2.3-559) 14.8 (2.4-26) 17.0 (3.7-26)</td>
</tr>
<tr>
<td>FLT1, median (range)</td>
<td>497</td>
<td>182 (48-97)</td>
</tr>
<tr>
<td>IGHV, (D) IGHV, (BVM, long) median</td>
<td>493</td>
<td>486 (201-1230)</td>
</tr>
<tr>
<td>Categorical Characteristic</td>
<td>n</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Age Stage</td>
<td>468</td>
<td>16 (3.4) 61 (13) 90 (19)</td>
</tr>
<tr>
<td>FR</td>
<td>525 (87) 75 (14)</td>
<td></td>
</tr>
<tr>
<td>Unmutated</td>
<td>485</td>
<td>180 (47) 205 (53)</td>
</tr>
<tr>
<td>Mutated</td>
<td>243 (42) 149 (34)</td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td>499</td>
<td>33 (7) 75 (15) 78 (16) 180 (20) 208 (42)</td>
</tr>
<tr>
<td>Del17p</td>
<td>33 (7) 75 (15) 78 (16) 180 (20) 208 (42)</td>
<td></td>
</tr>
<tr>
<td>Del17p</td>
<td>12 (3) 24 (5) 30 (6) 90 (19)</td>
<td></td>
</tr>
<tr>
<td>Del11q</td>
<td>10 (3) 20 (4) 30 (6) 90 (19)</td>
<td></td>
</tr>
<tr>
<td>Complex - Karyotype</td>
<td>591</td>
<td>35 (7) 75 (15) 78 (16) 180 (20) 208 (42)</td>
</tr>
<tr>
<td>Complex2</td>
<td>16 (3) 32 (6) 60 (12)</td>
<td></td>
</tr>
<tr>
<td>Simple</td>
<td>83 (17) 166 (34)</td>
<td></td>
</tr>
<tr>
<td>Diploid</td>
<td>347 (69)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

Aims: The aim of this study is to report the incidence and the impact of CpG-stimulated karyotype in the treatment of naive CLL.

Methods: We evaluated 501 treatment-naïve patients with CLL at MDACC between July 2013 and June 2016. CpG-stimulated metaphase karyotype of CLL cells from blood or bone marrow was performed by culture of mononuclear cells for 72 hrs in media containing CpG-865 (20ug/ml), phorbol 12-myristate 13-acetate (PMA; 0.04ug/ml) and Pokeweed mitogen (PWM; 0.1ug/ml). Banding and analyses were by standard laboratory procedures. Twenty metaphases were analyzed per culture and patients were categorized as having diploid karyotype, a single, 2 or 3 or more(complex) clonal chromosome abnormalities present in more than 1 metaphase by CpG-stimulated karyotype. The frequency and distribution of chromosome abnormalities with other prognostic factors and time-to-first treatment from diagnosis (TTFT) were analyzed (Table + Figure).

Results: The majority (69%) of patients had diploid cytogenetics. Higher-risk prognostic features such as del17p, del11q, unmutatedIGHV and ZAP70 expression were associated with presence of complex karyotype abnormalities. Shorter TTFT from diagnosis was associated with 1, 2, and complex clonal chromosome abnormalities compared to diploid karyotype (p<0.0001). A model was developed, which identified patient characteristics independently associated with shorter TTFT including: 1 or more clonal chromosome abnormality by CpG stimulated karyotype; unmutatedIGHV; 3 involved lymph node sites; and CD38 expression (>30%).

Summary/Conclusions: 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 TTFT. Models for TTFT may be useful in identifying patients at high-risk for needing treatment sooner and thereby useful for early intervention clinical trials.

E1017

COMPARISON OF THE CHRONIC LYMPHOCYTIC LEUKEMIA INTERNATIONAL PROGNOSTIC INDEX (CLL-IPI) WITH THE BARCELONA-BRNO PROGNOSTIC MODEL: ANALYSIS OF 1299 NEWLY DIAGNOSED CASES


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Background: In the last two decades, a plethora of clinical, serological and biological markers have been identified that are significantly associated with the prognosis of chronic lymphocytic leukemia (CLL) patients. Recent research has focused on the development of scoring systems capable of integrating the major prognostic parameters. A recent prognostic index called CLL International Prognostic Index (CLL-IPI), built on clinical, serological, and biological parameters (TP53 deletion and/or mutation, IGHV mutational status, β2M, clinical stage, and CD38 expression) has been proposed and validated. Recently, a CLL International Prognostic Index- naïve patient group, with the aim of simplifying the CLL-IPI, proposed a prognostic model comprising only two biomarkers (IGHV mutational status and FISH cytogenetics).

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 hybridisation (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 models were calculated using the methods proposed. The accuracy of the prognostic models was assessed by the Harrell C index (an index of discrimination), the explained variation in mortality (an index combining discrimination and 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-, 16.2% as high-, and 3.8% as very high-risk. The 5-year OS probabilities were 95% for low-risk, 89.9% for intermediate-risk, 70.1% for high-risk, and 32.8% for very high-risk cases (P<0.0001; Harrell C index=73%;P<0.001) (Figure 1A). According to the Barcelona-Brno prognostic model, 59.1% of patients were classified as low-, 31.8% as intermediate-, and 10.1% as high-risk. The 5-year OS probabilities were 92.2% for low-risk, 83.6% for intermediate-risk, and 68.2% for high-risk cases (P<0.0001; Harrell C index=65%;P<0.001) (Figure 1B). The AIC showed the superiority of the CLL-IPI compared to the Barcelona-Brno prognostic model in predicting OS (CLL-IPI, AIC=3432.167 versus Barcelona-Brno prognostic model, AIC=3549.492). According to the CLL-IPI, predicted variation in mortality provided by the Barcelona-Brno prognostic model was 42% (P<0.001), a figure higher than that due to the Barcelona-Brno prognostic model
Individuals who had received vaccination against Influenza and/or Pneumococcus were excluded from the analysis of the immunoglobulin-specific titers against S. pneumoniae (38.8% vs 40.6%, P>0.05). Individuals who had received vaccination against other bacterial infections were not different between groups: 76.9% vs 78.4% for CMV (P>0.05) and 70.4% vs 72.1% for EBV (P>0.05).

Methods: 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, the 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 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+AnnexinV+ 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 not 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 is planned.

E1019 INCREASED VIRUS-SPECIFIC IMMUNE RESPONSES PARALLELED BY A PNEUMOCOCCUS-SPECIFIC IMMUNODEFICIENCY STATE AND HYPOGAMMAGLOBULINEMIA: ALREADY EMERGE IN HIGH-COUNT MONOCLONAL B LYMPHOCYTOYSIS PRIOR TO CLL

Background: Patients diagnosed with chronic lymphocytic leukemia (CLL) 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. Aim: To evaluate the status of the humoral immune response in CLL at different disease stages, as well as in pre-lymphemic MBL and in MBL low count (MBLlo) 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 MBLlo, 29 CLl-like MBLhi and 58 MBLlo cases (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 isoforms and specific immunoglobulins against CMV (strain AD169), influenza virus (A/Brisbane/10/07), and pneumococcus 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 pathogens, respectively. Plasma CMV and EBV DNA load levels were measured using the quantitative PCR methods.

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 extent 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 3/177 individuals (1 MBL2 and 2 CLL- were found to be positive (below the limit of quantitation), while EBV DNA load was detected in plasma from 7/191 (all being Binet A CLL) at median levels of 3.6 copies/ul. In contrast to the virus-specific IgGs, IgG plasma levels against S.pneumoniae progressively diminished through progression of the disease, in parallel to the overall gammaglobulin levels.

Summary/Conclusions: Both MBL2 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 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.

E1020
AN EXTENSIVE MOLECULAR CYTOGENETIC CHARACTERIZATION IN HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA IDENTIFIES KARYOTYPE ABERRATIONS AND TP53 DISRUPTION AS PREDICTORS OF OUTCOME AND CHEMOREFRACTORYNESS
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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 11q22/17p13 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 Pgo-oligonucleotide DSP30. Each patient was categorized according to the following classification: favorable group (isolated 13q14 deletion or normal karyotype), unfavorable group (deletions of 11q22 or 17p13, 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 mutational status, \( \beta 2\)-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 external 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 (4553 or 73.7%) came from studies of external validation of CLL-IPI while 17% (1192) and 8.5% (576) had been used to generate (training sets) and to internally validate the model. Patient distribution into the four risk categories of CLL-IPI was heterogeneous thus reflecting the CLL phase (ie, 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 “Q” or “I2” test to assess the heterogeneity across different studies. The 5-year survival probability was 91% for low-risk group (95% CI, 90-91%), Q=55.2; P< 0.001; I^2 =87%), 89% (95% CI, 79-82%); Q=49.36; P< 0.001; I^2 =86%), 60% for high-risk group (95% CI, 57-62%; Q=42.78; P< 0.001; I^2 =84%) and 32% for very high-risk group (95% CI, 27-38%; Q=18.1; P=0.01; I^2 =67%).

Figure 1.

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 presents 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 idelisib 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
A. Peters1,*, J. Wiemikowski2, S. Barker3, M. Mahler4, S. Luke5, A. Lodick5

haematologica | 2017; 102(s2) | 419

Madrid, Spain, June 22 – 25, 2017
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 (McCue DA, et al. Pharmacotherapy. 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. A patient adherence 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 score of 92.3%, as compared with 63.5% for patients who did not receive nurse coaching (95% CI, 75.3-91.6; p < 0.0001). At 3 months the adherence rates were 89.9 vs 60.8% (95% CI, 75.5-91.4; p < 0.0001). By 9 months, adherence rates were 81.7% vs 71.1% (95% CI, 4.4 to 28.4; p = 0.141). 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. E1024 SINGLE-AGENT IBRUTINIB VS REAL WORLD TREATMENT FOR PATIENTS WITH TREATMENT-NAIVE (TN) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): AN ADJUSTED COMPARISON OF RESONATE-2™ WITH THE CLLEAR AND LYON-SUD DATABASES M. Doubek1*, E. Bachy2, M. Spacek3, L. Basseggi4, R. Besson5, J. Diels6, J. Garside7, N. Healy8, W. Iraqui8, E. Callet-Bauchu2, L. Smolej9, G. Salles2

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Background: The phase 3 RESONATE-2™ study demonstrated significant improvement of progression-free survival (PFS) and overall survival (OS) with ibrutinib (ibr) vs chlorambucil (ch) in TN (aged ≥65 years) CLL patients. In the absence of direct comparison of single-agent ibr with other frequently utilized regimens in this patient population, this retrospective outcome study from the CLCLEAR (Chronic Lymphocytic Leukemia Registry) and Lyon-Sud databases may provide insight into the impact of ibr on patient outcomes, especially in CLL patients with del(17p) who receive ibr as monotherapy in the absence of standard of care as observed in clinical practice can provide useful insights on the relative efficacy of ibr. Aims: To investigate the relative treatment effect on PFS and OS for ibr vs real world (RW) treatment in daily clinical practice in TN CLL patients by adjusting for baseline characteristics from patient-level data of RESONATE-2™ vs real world treatment (RW) in the CLCLEAR and Lyon-Sud databases. Methods: CLCLEAR holds medical records for CLL patients from seven academic centers across the Czech Republic. Lyon-Sud database holds medical records for CLL patients from the clinical French hospital Lyon-Sud. Patients initiated on CLL frontline therapy were selected from CLCLEAR and Lyon-Sud using the same inclusion-exclusion criteria as for RESONATE-2 (excluding patients with age<65 and with del17p positive status). PFS and OS were com-
pared between ibru and RW treatment using patient-level data from RESONATE-2™ (n=136) and pooled patient-level data from the two cohorts. To adjust for differences in patient characteristics between the trial population and the general population, independent prognostic factors for OS were included as covariates.

**Results:** Median age at treatment initiation for CLEARLE (n=1418) and Lyon-Sud (n=110) was 73 and 71 years, respectively, vs 73 for ibru patients from RESONATE-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. Fluorarabine/cyclophosphamide/rituximab (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, advanced disease stage and del11q positive status were independent risk factors 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 0.53 [0.13-0.83] (BR) for OS (Figure 1). Estimates of HR vs regimens in the cohorts were consistent across both databases.

**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 OS, 2.7-fold improvement in PFS, and 1.9-fold improvement in TFS. 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 OS, 2.7-fold improvement in PFS, and 1.9-fold improvement in TFS. This adjusted comparison of patient-level data from RESONATE-2™. The proportion of male patients was 63% in CLEARLE and 57% in Lyon-Sud vs 65% in RESONATE-2™. The median age at treatment initiation for CLEARLE (n=1418) and Lyon-Sud (n=110) was 73 and 71 years, respectively, vs 73 for ibru patients from RESONATE-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™.
(MIFIR) was calculated as a relative expression between MFIR positive popula-
tion and MFIR negative population. Multivariate analysis was used to assess sta-
tistical significant differences in accuracy among individual markers and scor-
ing systems. The treating physician made the final diagnosis of the different
B-cell malignancies according to IWCLL and WHO criteria. Logistic regression
including sensitivity, specificity and accuracy values, were used to evaluate statistical
differences in diagnostic precision between different combinations of
markers as well as individual markers.

**Results:** Flow cytometry analysis was performed in 99 patients, including 62
cases with a diagnosis of CLL (62.6%) and 37 cases with a “non-CLL” diagnosis
(37.4%). Matutes score was 4-5 in all CLL cases and ≤3 in “non-CLL” cases.
CD20, CD79a and C5 were the most consensual markers for CLL (90.3%, 96.8%
and 100.0% of sensitivity respectively). Moreover, CD79b and FMC7 had a good
discriminative value (80-85% sensitivity). For “non-CLL” cases the most reliable
markers were SmIg, FCMD7 and also CD20. The analysis of the accuracy is shown in the table. Of note, CD20 as a single marker was found to be a reliable marker for distinguishing CLL and “non-CLL” cases
(90.9%; p<0.001; 90.3% sensitivity, 91.9% specificity) showing a significantly
higher accuracy than CD5, CD23 and SmIg as individual markers (p<0.001).
The accuracy of CD20 did not vary when comparing% of positive cells and
MIFIR. In contrast, the accuracy for MIFIR significantly increased from 67.7% to
78.5% when comparing MIFIR values (according to the cut-off established by ROC
curves), being lower in CLL than in “non-CLL” cases (71.0% vs 86.5%,
p<0.001). Finally, the addition of CD20 to the Matutes score and using a
cut off ≥4, improved its accuracy from 88.9% (95% CI: 88.2-95.6) to 98.0%
(95% CI: 94.7-100.0) and showed a better sensitivity.

<table>
<thead>
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<th>Marker</th>
<th>Score prediction</th>
<th>CLL ≤4 (Non-CLL)</th>
<th>CLL ≥5 (%)</th>
<th>p-value vs ≤4</th>
<th>p-value vs ≥5</th>
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<tr>
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<td>0.4</td>
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<tr>
<td>CD20</td>
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<td>0.7</td>
<td>0.001</td>
<td>0.005</td>
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<tr>
<td>CD79a</td>
<td>Positive</td>
<td>0.6</td>
<td>0.7</td>
<td>0.001</td>
<td>0.005</td>
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<tr>
<td>C5</td>
<td>Positive</td>
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<td>0.7</td>
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</tr>
<tr>
<td>SmIg</td>
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<td>0.7</td>
<td>0.001</td>
<td>0.005</td>
</tr>
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</table>

**Table 1.**

Summary/Conclusions: These results confirm CD20 as a valuable marker in
the diagnosis of CLL.

**E1027**

COMPARISON OF CHROMOSOME BANDING ANALYSIS AND GENOMIC
MICROARRAY TECHNIQUES FOR THE DETECTION OF COMPLEX
KARYOTYPES IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Hospital Universitario Ramón y Cajal, Madrid, 4Servicio de Hematología, Hospital
Universitario de la Santa Creu i Sant Pau, Barcelona, 5Servicio de Hematología,
Consorcio Hospital General Universitario, Valencia, 6Servicio de Hematología,
Hospital de Cruces, Bilbao, 7Laboratorio de Citogenética y Servicio de Hematología,
Hospital Vall d’Hebron, Barcelona, 8Servicio de Hematología, Hospital
Universitat Marqués, València, Santander, 9Servei de Hematologia, Hospital
del Mar, Barcelona, Spain

**Background:** Well-established poor prognostic factors 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
chains, 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:** Patients main characteristics are detailed [N=147, M/F:75/72, 111 in
early stage disease, 17p in 11 patients and Del17q in 15]. Median age was

---

**Figure 1.**

Results: Median patients characteristics are detailed [N=147, M/F:75/72, 111 in
early stage disease, 17p in 11 patients and Del17q in 15]. Median age was
PLATELET FUNCTION ASSAYS FOR STRATIFICATION OF BLEEDING RISKS IN CLL PATIENTS ON IBRUTINIB TREATMENT

E. Nikitin1,*, E. Dmitrieva1, A. Ignatova2, A. Poletaev2, D. Polokhov2, A. Fedotov2, RISKS IN CLL PATIENTS ON IBRUTINIB TREATMENT PLATELET FUNCTION ASSAYS FOR STRATIFICATION OF BLEEDING E1029

Aims: To investigate platelet function activity in CLL patients before and after ibrutinib treatment.

Methods: We collected platelet function data from 57 patients treated with ibrutinib. A total of 935 samples were analyzed using classical aggregation assays and the flow cytometry-based PFA-100 system. The primary endpoint was the change in platelet function after ibrutinib treatment.

Results: The median time to treatment completion was 3 months (range 1-6). Del17p or TP53 mutation was found in 11 (25%) patients. Only 1 patient received anticoagulant and antiplatelet drugs. Median duration of treatment was 3 (range, 1-6). Del17p or TP53 mutation was found in 11 (25%) patients.

Discussion: Our study showed that ibrutinib treatment systematically induced a modification of the sFLC K/L ratio. Interestingly, after treatment completion, the persistence of an abnormal sFLC K/L ratio was associated with positive MRD determined by FCM with a 82% specificity and a 95% positive predictive value. Moreover, median time to treatment completion was 3 (range, 1-6). Del17p or TP53 mutation was found in 11 (25%) patients.

Summary/Conclusions: Both classic aggregation assays and flow-cytometry-based techniques demonstrate impaired platelet function in the bleeding CLL patients compared with non-bleeding ones. The level of integrin activation possibly prevents severe bleeding on ibrutinib. Here we investigate platelet function activity in CLL patients before and after ibrutinib treatment.

Background: Management of chronic lymphocytic leukemia (CLL) dramatically improved since the introduction of novel therapies. Rarely patients require treatment at diagnosis and approximately a third of patients will never require therapy. Predictive and prognostic factors are well known (IGHV, del11q, del17p, TP53); CLL-IPI score including age, clinical stage, beta2-microglobulin, IGHV and deletion 17p and/or TP53 mutation has been recently validated. It identifies 4 risk groups with significantly different time to first treatment (TTFT) and overall survival (OS). Hypogammaglobulinemia (HYPO) is a typical feature of CLL, with an incidence of 20-60% at diagnosis and a relationship with infections occurrence. Prognostic significance of HYPO at diagnosis has not been extensively evaluated in terms of OS and TTFT. Only IgG serum levels have been reported to be associated with TTFT but no data are available on other immunoglobulin classes (Ig).

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) levels. IgG, IgM and IgA at diagnosis and calculated CLL-IPI. HYPO was defined basing on our laboratory cut-offs (IgG 70mg/dl, IgG 700mg/dl, IgG 40mg/dl).

Results: Among 698 patients assessed, 410 cases were evaluable for Ig values 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 180mg/dl for IgM (Figure 1, D-E-F). Considering Ig deficit, patients presented IPI 0-1, 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. However, 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 prognosis.

Figure 1. 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 prognosis.
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 lower lymphocytes in initial clinical stage (p<0.001), higher BLC (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<0.001) 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 (mainly, chemotherapy and immunotherapy) did not differ more frequently treated with alkylating agents than purine analogues. Conversely, patients treated due to LM had more frequent adverse prognostic characteristics, such as higher ZAP70 expression, unmutated IGHV genes, 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<0.001) 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 (mainly, chemotherapy and immunotherapy) did not differ more frequently treated with alkylating agents than purine analogues.

Summary/Conclusions: All ITT 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 ITT 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 infiltrative cytopenia, progressive splenomegaly or lymphadenopathy, short lymphocyte doubling time (LDT), B symptoms and/or refractory disease (RD). 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 clinic-biological characteristics and prognosis of CLL patients according to the criteria that prompted the initiation of first-line treatment.

Methods: Retrospective analysis of 1,405 consecutive patients with CLL who received first-line therapy from 1978 to 2014 and had their indication(s) for treatment (ITT) recorded. Massive/progressive lymphadenopathy and massive/progressive splenomegaly were grouped together as lymphoid mass (LM), Infiltrative anemia and thrombocytopenia were categorized as marrow failure (MF). As 505 patients initiated therapy due to LM or RD, we decided to focus on these two groups. Patients whose ITT 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 (range 1-433). Half of the patients had a single ITT, while the other half had two or more. ITT were LM in 72% patients, MF in 31%, short LDT in 29%, B symptoms in 19%, and IC in 3%. Compared to patients from the LM group, patients from the MF group were significantly older, had a significantly higher β2-microglobulin level (probably due to an age-related impaired renal function), and were more frequently treated with alkylating agents than purine analogues. Conversely, patients treated due to LM had more frequent adverse prognostic characteristics, such as higher ZAP70 expression, unmutated IGHV genes, and 11q deletion. The median OS of the entire population was 77 months (95% confidence interval [CI]=71-83) from first-line therapy and 108 months (95% CI=102-118) from diagnosis. Indication for treatment was significantly associated (p<0.001) with a shorter OS from first-line therapy: 63 months in the MF group (95 CI: 48-72), compared to 89 months in the LM group (95% CI: 80-106). This association remained significant after adjusting for age and β2-microglobulin concentration.

Figure 1.
Summary/Conclusions: All ITT 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 ITT 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 deletion of all or part of the chromosome 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.

References:

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 410 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 nonclonal 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 nonclonal 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 nonclonal 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 nonclonal 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 mislabelling it as a secondary minor clone.

E1034 PRIMARY PEGFILGRASTIM PROPHYLAXIS VERSUS FILGRASTIM GIVEN “ON DEMAND” FOR CLADRIBINE-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 neutropenic fever (NF). Based on the script data: 71% of patients experienced grade 4 neutropenia (absolute neutrophil count [ANC] <500x10^9/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 following “on demand” 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 intravenous 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 <1000x10^9/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 ≥1000x10^9/L.

Results: Mean follow up was 7.5 years (0.1-40), with 5 and 10 years’ survival of 96% and 90.62% 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 among 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 10^9/L and ANC <0.310^9/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 filgastrim *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 (PPP). 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.

<table>
<thead>
<tr>
<th>CR IR PPPM</th>
<th>Non-CR IR PPPM</th>
<th>Unadjusted IR</th>
<th>Adjusted IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.006</td>
<td>0.003</td>
<td>3.30 (1.69-6.21)</td>
<td>2.44 (1.13-5.42)</td>
</tr>
</tbody>
</table>

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 0 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 confounders, the incidence of all-cause hospitalization was 2.4 times higher for patients who did not achieve CR compared to those who did (IRR=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 of care for CLL pts with AIHA, especially for the cohort of pts with comorbidities. R 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 NAIVE 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 6-week course of treatment of 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 IV of 375mg/m2 once weekly for 6 consec- utive 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 50x10^3/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 median WBC and ALC counts of treatment naïve CLL pts by analyzing concomitantly its efficacy and safety as a single agent in CLL therapy

Results: 13 pts (86.7%) achieved PR and 1 CR (6%). All pts with advanced disease also responded entering PR. Resolution of splenomegaly was documented in both splenomegalic 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 infusion related reaction in a patient with WBC>400x10^9/L without need for hospitalization. None of the pts experienced severe tumor lysis syndrome, pulmonary insufficiency, myelosuppression or opportunistic infections.

Table 1.

<table>
<thead>
<tr>
<th>WBC (x10^3)</th>
<th>67.3 (6.9-521.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before R</td>
<td></td>
</tr>
<tr>
<td>After R</td>
<td></td>
</tr>
<tr>
<td>6.7R</td>
<td></td>
</tr>
</tbody>
</table>

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 responsi

E1037
ATTAINMENT OF COMPLETE REMISSION IS SIGNIFICANTLY ASSOCIATED WITH LONGER 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/without minimal residual disease in first-line chemotherapy 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, 1p7 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 or 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 con- 353

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.

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.

Figure 1.
E1038

APLICATION OF THE CLL-IPI AND THE MDACC PRGNOSTIC INDEXES IN A LOCAL COHORT OF CLL PATIENTS

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1Hospital Universitario Infanta Leonor, MADRID, 2IBSAL, IBMCC, Centro de Investigación del Cáncer, Universidad de Salamanca-CSIC, Salamanca, Spain

Background: New prognostic scores have been developed in order to better discriminate the clinical course of CLL patients, along with Rai and Binet clinical staging systems. These scores, such as that proposed by the MDACC group, and recently the CLL-IPI combine clinical and biological variables with prognostic value.

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 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 ALC (p=0.001), shorter LDT (p=0.001), and more often anemia (p=0.2), 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 34%), purine analogs (4% vs 27%), CIT (0% vs 31%), other (5% vs 8%) (p<0.001). The response rate was lower in patients diagnosed before 1995 (57% with 9% CR vs 61%, 36% CR, 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 broken down by clinical stage was: 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 8.3 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 after 1995 (p<0.001). In patients diagnosed before 1995, the number of subjects with available FISH was too small for a meaningful analysis. In multivariate analysis (age >70 years, advanced clinical stage short LDT increased B2M, diagnosis before 1995 only) HR 2.7 (95% CI: 2.1-3.4), p<0.001, LDT (HR 2.5 (1.9-3.2), p<0.001) and B2M (HR 2.8 (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 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.
patients (27.4%). Overall, 80.3% of FAS patients did not experience therapeutic failure and 85.9% did not experience disease progression during the 2-year observation period. By the end of the study, median PFS had not been reached, 2-year PFS rate was estimated as 85.9%. Improvements from baseline were observed after 6 cycles of treatment across all EQ-5D domains. No relapses or deaths occurred in the FAS; however, 2 subjects in the Safety Population experienced fatal serious ADRs (myocardial infarction [n=1]; acute pneumonia, infections and toxic shock, and atrial fibrillation [n=1]). In concurrence with the Phase 3 trial results, hematologic disorders (19.9%; anemia, neutropenia, thrombocytopenia), most of which were Grade ≤2 in severity, were the most common ADRs (Safety Population; Table 1).

Table 1. Hematologic ADRs by CTCAE Grade.

<table>
<thead>
<tr>
<th>Hematologic ADR</th>
<th>Grade 1 (%)</th>
<th>Grade 2 (%)</th>
<th>Grade 3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>49.5</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>21.7</td>
<td>17.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2.3</td>
<td>0.3</td>
<td>0.0</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.

Chronic myeloid leukemia - Biology

E1041

MUTAGENESIS OF BCR-ABL1 IS REQUIRED FOR RESISTANCE DEVELOPMENT IN DE NOVO CHRONIC MYELOID LEUKEMIA KCL-22 CELLS BUT NOT IN RELAPSED KCL-22 CELLS EXPRESSING BCR-ABL1 INDEPENDENT RESISTANCE

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Background: BCR-ABL1 kinase domain (KD) mutations are an important mechanism of resistance of chronic myeloid leukemia (CML) patients developing during the tyrosine kinase inhibitors (TKI) treatment. However, mechanisms underlying KD mutation acquisition in TKI-resistant CML cells are not yet well understood.

Aims: We studied an acquisition of mutations in the KD after an exposure of de novo and relapsed (grown in optimal growing medium for 24 months) KCL-22 cells to imatinib (IM). In addition, we examined kinetics of mutated subclones in established IM-resistant KCL-22R culture after dose-reduction of IM. We also studied changes in the expression profile of KCL-22 cultures early after exposure to IM.

Methods: The occurrence and kinetics of expansion of BCR-ABL1 mutant subclones were studied using next-generation deep sequencing in KCL-22 cells treated with 0.4 µM IM and in established IM-resistant KCL-22R cells at 4 µM IM. In other set of experiments, KCL-22R cells were sorted according to the CD38 expression to explore whether CD38 is associated with the acquisition of BCR-ABL1 mutations as suggested by Wang et al. (2014). A protein array was used allowing analysis of 576 proteins per sample. DNA damage pathway-RT Profiler PCR arrays were applied for gene expression analysis.

Results: No BCR-ABL1 KD mutations were detected in de novo untreated KCL-22 cells, however T315I and E255K appeared after the exposure of the cells to 0.4 µM IM. PCR array revealed increased expression of SUMO 1 ligase and ERCC2 involved in the nucleotide excision repair pathway. Notably, we also found a significant decrease of G2/M-checkpoint protein GAID45a whose deficiency is associated with mutagenesis (Hollandar et al., 2001). During the first culture period, T315I slowly emerged whereas E255K was not detectable. Later, E255K-bearing cells also became detectable and increased over time. A similar time-dependent expansion of mutant-bearing sub-clones was seen in the KCL-22R cells growing at 4 µM IM. Interestingly, a mutant-clone switch from T315I to E255K in KCL-22R was accelerated after IM reduction from 4 µM to 1 or 2 µM. Moreover, the emerging of E255K sub-clones was accompanied by rapid decrease of CD38 expression in KCL-22R cells. Profiling of transitional KCL-22R culture, carrying both T315I and E255K sub-clones, revealed that T315I transcripts were expressed only in the CD38+ subpopulation, while E255K was detected only in CD38- cells. Unlike to de novo KCL-22 cells, BCR-ABL1 mutations were repeatedly not detected in relapsed KCL-22R cells, until 60 days after the cells exposure to 0.4 µM IM. Neither BCR-ABL1 upregulation nor gene amplification was detected in these cells. We identified considerably upregulated (D7, DTX3, ETV6, GLUL, HCLS1, HIF1α, IGF1R, MAP2K7, MYH11, TP53) or downregulated (BAD, BID, MCL2 NOTCH3, PDKPI1) proteins early, 4 weeks after the exposure to IM. Increased expressions of HIF1α and IGF1R proteins are known to ensure proliferation, while decreased expressions of pro-apoptotic proteins BAD and BID enhance survival of CML cells in the presence of TKIs.

Summary/Conclusions: Our observation suggests the ability of KCL-22 cells to survive and proliferate early after the exposure to IM. BCR-ABL1 mutations development seems to be related to a mutagenesis of imatinib in de novo KCL-22 cells, but not on relapsed KCL-22 cells that activated signaling pathways ensuring their survival and growing in the presence of tyrosine kinases inhibitor.

Supported by the project no. 00023736 and AZV 15-31540A of MZCR and ERDF OPPK CZ.2.16/3.1.00/28007.

E1042

FLOW-CYTOMETRY DETECTION OF CD26+ LEUKEMIA STEM CELLS IN PERIPHERAL BLOOD: A SIMPLE AND RAPID NEW DIAGNOSTIC TOOL FOR CHRONIC MYELOID LEUKEMIA

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Background: BCR-ABL1 kinase domain (KD) mutations are an important mechanism of resistance of chronic myeloid leukemia (CML) patients developing during the tyrosine kinase inhibitors (TKI) treatment. However, mechanisms underlying KD mutation acquisition in TKI-resistant CML cells are not yet well understood.

Aims: We studied an acquisition of mutations in the KD after an exposure of de novo and relapsed (grown in optimal growing medium for 24 months) KCL-22 cells to imatinib (IM). In addition, we examined kinetics of mutated subclones in established IM-resistant KCL-22R culture after dose-reduction of IM. We also studied changes in the expression profile of KCL-22 cultures early after exposure to IM.

Methods: The occurrence and kinetics of expansion of BCR-ABL1 mutant subclones were studied using next-generation deep sequencing in KCL-22 cells treated with 0.4 µM IM and in established IM-resistant KCL-22R cells at 4 µM IM. In other set of experiments, KCL-22R cells were sorted according to the CD38 expression to explore whether CD38 is associated with the acquisition of BCR-ABL1 mutations as suggested by Wang et al. (2014). A protein array was used allowing analysis of 576 proteins per sample. DNA damage pathway-RT Profiler PCR arrays were applied for gene expression analysis.

Results: No BCR-ABL1 KD mutations were detected in de novo untreated KCL-22 cells, however T315I and E255K appeared after the exposure of the cells to 0.4 µM IM. PCR array revealed increased expression of SUMO 1 ligase and ERCC2 involved in the nucleotide excision repair pathway. Notably, we also found a significant decrease of G2/M-checkpoint protein GAID45a whose deficiency is associated with mutagenesis (Hollandar et al., 2001). During the first culture period, T315I slowly emerged whereas E255K was not detectable. Later, E255K-bearing cells also became detectable and increased over time. A similar time-dependent expansion of mutant-bearing sub-clones was seen in the KCL-22R cells growing at 4 µM IM. Interestingly, a mutant-clone switch from T315I to E255K in KCL-22R was accelerated after IM reduction from 4 µM to 1 or 2 µM. Moreover, the emerging of E255K sub-clones was accompanied by rapid decrease of CD38 expression in KCL-22R cells. Profiling of transitional KCL-22R culture, carrying both T315I and E255K sub-clones, revealed that T315I transcripts were expressed only in the CD38+ subpopulation, while E255K was detected only in CD38- cells. Unlike to de novo KCL-22 cells, BCR-ABL1 mutations were repeatedly not detected in relapsed KCL-22 cells, until 60 days after the cells exposure to 0.4 µM IM. Neither BCR-ABL1 upregulation nor gene amplification was detected in these cells. We identified considerably upregulated (D7, DTX3, ETV6, GLUL, HCLS1, HIF1α, IGF1R, MAP2K7, MYH11, TP53) or downregulated (BAD, BID, MCL2 NOTCH3, PDKPI1) proteins early, 4 weeks after the exposure to IM. Increased expressions of HIF1α and IGF1R proteins are known to ensure proliferation, while decreased expressions of pro-apoptotic proteins BAD and BID enhance survival of CML cells in the presence of TKIs.

Summary/Conclusions: Our observation suggests the ability of KCL-22 cells to survive and proliferate early after the exposure to IM. BCR-ABL1 mutations development seems to be related to a mutagenesis of imatinib in de novo KCL-22 cells, but not on relapsed KCL-22 cells that activated signaling pathways ensuring their survival and growing in the presence of tyrosine kinases inhibitor.

Supported by the project no. 00023736 and AZV 15-31540A of MZCR and ERDF OPPK CZ.2.16/3.1.00/28007.
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-ABL1 fusion by FISH or BCR-ABL1 rearrangement by RT-PCR. In clinical practice, at the earliest, 24-72 hrs are needed to confirm CML by any of these assays. Late characterization of CML leukemia stem cells (LSCs) from BM samples by BM CML patients (pts) showed a specific co-expression of dipeptidyl peptidase IV (CD26) within the CD34+/CD38−/Lin− stem cell fraction and CD26 appeared a robust biomarker showing a specific co-expression of dipeptidyl peptidase IV (CD26) within the CD34+/CD38−/Lin− stem cell fraction and CD26 appeared a robust biomarker.

Summary/Conclusions: Flow-cytometry also in PB during treatment with tyrosine kinase inhibitors.

Aims: We investigated accuracy and specificity of flow cytometry PB CD34+/CD38−/CD26+ LSCs identification as a new tool for the diagnosis of CML.

Methods: Patients with clinical suspicion of CML entered the study after written informed consent and all were evaluated for CD26-LSCs, cytogenetics, FISH and/or BCR-ABL1 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. 2.0x10⁶ leukocytes were incubated with CD Phenotypic antibodies (BD Biosciences). CD26+ cells were 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 52x10⁹/L (range 5-409x10⁹/L). In 83/107 (77.5%) pts we showed CD34+/CD38−/CD26+ LSCs in PB and in 83/107 (100%) the diagnosis of CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/L µL was 14 (range 0.27-698) and a positive correlation with leukocyte count (p<0.01) was found. In 53/107 (49.5%) pts analysis was performed contextually in BM samples. On average, CD34+/CD38−/CD26+ population was gated applying a narrow gate excluding all CD38+ cells (Fig.1).

Figure 1.

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-treated 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.
E1045
MAINTENANCE OF LEUKAEMOGENIC POTENTIAL OF BCR/ABL+ CELLS REQUIRES PAK2 BUT NOT PAK1
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Background: Transcribed ultraconserved regions (T-UCR) are a novel class of long noncoding RNAs. Many classes of noncoding RNAs have been implicated in human tumorigenesis. In addition to the different expression profiles of T-UCRs that could be used to distinguish human leukemias and cancers, there have also been reported to have direct interactions with miRNA with an important regulatory effect in disease development such as chronic myeloid leukemia (CML).

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 Benjamin-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.153 (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.294 (p<0.05) and imatinib doses, uc.4 (p<0.05) and uc.3 (p<0.05) inversely correlated with 400 and 800mg daily, respectively. Molecular expression 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:miRNA 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 T-UCR:miRNA 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.

Financial Support: FEDER (Programa Operacional Factores de Competitividad – COMPETE) and FCT (Fundaçao para a Ciencia e Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.

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 non-hematologic 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. Synthesis of 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-218, 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: in 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.
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.

Financial Support: FEDER (Programa Operacional Factores de Competitividade – COMPETE) and FCT (Fundação para a Ciência e a Tecnologia) through project PEst-C/SAU/LA0001/2013-2014.

**E1048**

**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:** In order to better understand the role of the PcGs genes in CML patients receiving TKIs, we analyzed the expression of 88 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%/IS, according to the European guidelines, and the expression of the chosen 88 PcGs by real-time PCR (PrimePCR pathway kit, Biorad, Milan, Italy) at diagnosis and after 6 months of treatment. Expression values were calculated using the 2-DDCt 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) HLF, 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 TKI 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 pathogenic 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 responders. Five of them: rs116201358, rs17882014, rs4014596, rs52897880 and rs2274329 in 8A, HLA-DRB1, UNC93B1, APOH and CAT6 genes respectively, were present in responders; rs4945 in MFGE8 was present in failures. Mutations in HLA-DRB1 (rs17878951, rs11554462, c.239C>G), HLA-DRB5 (rs137883146), RPHN2 (rs193179333), CYP2F1 (rs116958555), KCNJ12 (rs76684759), FUT3 (rs151218854), BM1C (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 oncogene BCR-ABL on the 22q-chromosome. It is known that the protein products of the genes of cytokines ensure homeostasis at the cellular and tissue level, carrying out the metabolism of toxic compounds that can damage the genome of the cells. Present study evaluates the role of genotypes of A2425G polymorphism of CYP 1A1 gene in the development of a number of neoplastic diseases, including leukemia. In individuals with weakened functional genotypes of A2425G polymorphism of CYP 1A1 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 A2425G polymorphism of CYP 1A1 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 of 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 AmpE Sensor (Leucosis quautomom, M-1, A2425G FR(T) (InterLabServis, Russia). Testing A2425G polymorphism of CYP1A1 gene was performed on a programmable thermal cycler of the company “Applied Biosystems” (USA) using test systems company “Litech” (Russia) according to the manufacturer’s instructions. Statistical analysis of results was carried out using the statistical software package “2009 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; χ2=6.8; P=0.01; OR=2.0; 95% CI 1.17- 3.28). 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 (χ2=4.6; P=0.03; OR=1.8; 95% CI 1.046-3.166). The homozygous genotype G/G in the group of patients was significantly more compared to the control group (1.4% vs 0.0%), but due to the small number of carriers of this genotype differences in the results did not reach statistical significance (P=0.05). Functionally favorable genotype A/A was found with high frequency in a population-based sample of 86.6% vs 76.7% cases of patients. At the same time, the differences reached the threshold level of significance (χ2=26.0; P=0.01; OR=0.5; 95% CI 0.29-0.88), that is evidence of a favorable protective effect of this genotype against the development of CML.

Summary/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 (P<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.

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.

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 2° GTKIs (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: 2° 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...
in 1st line, 114 in 2nd, 56 in 3rd) only 13 had hematologic toxicity and 6 had to switch, 14 had pleural effusion grade III-IV and 9 had to switch. From 115 patients treated with nilotinib (49 in 1st line and 66 in 2nd) only 10 had hematologic toxicity and 10 switched treatment. Survival: Estimated survival by 10 years was 80%. Variables associated with survival: In the univariate survival analyses (log rank test) either from diagnosis, first therapy or first TKIs, the Sokal, Eutus, Euro and EUTOS LT scores, as well as age over 70y were the only statistically significant variables associated with survival. Hematological toxicity grade III-IV was associated with lower PFS or OS (figure 1). In the multivariate analysis (Cox model), only hematology toxicity grade III-IV and age over 70y were independent variables.

Summary/Conclusions: 1. These results show that the probability of survival by 10 years is roughly 80%, and extend the findings of our previous work showing that this probability is not different across different sequential treatments (imatinib 1st line or post-IFN, or switched to 2nd TKIs due to intolerance or failure) (1). This fact emphasizes the reserve 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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Methods: Treatment-naïve 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 retrospectively.

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 50mgw a safe and effective means of managing patients who experienced AEs in this 5-year retrospective analysis of DASISION. These results were consistent with previous reports and continued to show that efficacy was not affected by dose reductions 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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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 CYP2A5*3 (rs98964Q [rs776746]), ABCB1 [3435T>C (rs1045642)], ABCG2 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.

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 patients entered both trials, but safety data had not been collected in STAT1. In STAT2, patients entered both trials, but safety data had not been collected in STAT1 after entering STAT2 to avoid double counts. The PK/Px data of 147 of 154 patients were available and were evaluated in this study. Median trough concentrations of NIL were 1265 ng/ml at 1M, 1154 ng/ml at 3M, 974 ng/ml at 6M, 735 ng/ml at 12M, and 781 ng/ml at 24M. Although any-grade AEs were reported for patients in STAT1 and 55 patients in STAT2, the most common drug-related hematological and non-hematological AEs were elevated total bilirubin (28.6%), anemia (24.5%), elevated ALT (21.1%), and elevated AST (18.4%). The incidence of these AEs, except for anemia, was significantly associated with...
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 *11/*11 [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. As dasatinib is a novel 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 the observational, open-label study, 102 patients with CP-CML were enrolled to receive dasatinib at a dose of 100mg once daily. The primary end point was complete molecular response (CMR) by 18 months. Secondary end points including molecular response (MR) by 1, 3, 6, 12, 18, 24 month, time to and duration of MMR and CMR, and safety were tested. A receiver operating characteristic (ROC) curve from BCR-ABL1 transcript level on Day+28 was calculated to predict EMR and MMR at specific timepoints.

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%) of 102 patients were still on dasatinib treatment and 22 patients discontinued treatment because of drug failure (n=2) or for 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 79.5% respectively. Expected survival for 3L CP-CML patients prior to the availability of ponatinib in this study was 96% at 2 years, 90% at 3 years and 85% at 5 years.

Summary/Conclusions: Our study shows that VEMR at 1 month can be a strong predictor for further molecular responses as well as long-term outcome. Therefore it would be helpful to monitor BCR-ABL1 transcript level at 1 month in patients who treated with more potent TKIs.

E1055

SURVIVAL OUTCOMES IN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) RECEIVING THIRD- OR SUBSEQUENT LINE (3L) TREATMENT PRIOR TO THE AVAILABILITY OF PONATINIB

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Background: PACE was a phase 2 single-arm trial of ponatinib, a 3rd-generation tyrosine kinase inhibitor (TKI), in 449 highly-refractory patients with CML or Philadelphia-chromosome positive (Ph+) acute lymphocytic leukemia (ALL) or who had the BCR-ABL1 T315I mutation. Overall survival (OS) for 3L CP-CML patients in PACE at 1, 2, 3 and 4 years was estimated to be 99%, 93%, 88%, and 79%, respectively. Expected survival for 3L CP-CML patients prior to the availability of ponatinib has not been documented.

Aims: To estimate OS in patients with CP-CML receiving 3L treatment prior to ponatinib via a systematic literature review.

Methods: Studies were identified from a review by Lipton et al. (2015), updated with studies identified from searches of electronic databases (MEDLINE, EMBASE, Cochrane Libraries) and abstract databases of key conferences. Landmark and median survival were extracted from study reports. Pseudo-individual patient data (IPD) for survival outcomes were derived from digitized Kaplan-Meier (KM) survival curves then pooled and analyzed using KM methods.

Results: Fifty-five studies (717 patients) were identified that reported median, landmark, or KM curves for survival outcomes for CP-CML patients receiving 3L treatment without ponatinib. KM curves for OS were obtained for 6 arms (3 nilotinib and/or dasatinib; 3 other TKIs). OS at 1, 2 and 3 years based on the pooled IPD is reported in the Table. To avoid confounding of OS from post-progression treatment with ponatinib, 1 study was excluded that included follow-up after the date of ponatinib’s approval.

Table 1

<table>
<thead>
<tr>
<th>Time (years)</th>
<th>Pts</th>
<th>Arms</th>
<th>Probability (95%CI)</th>
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<tr>
<td>0</td>
<td>327</td>
<td>5</td>
<td>100% (95-100)</td>
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<td>1</td>
<td>257</td>
<td>5</td>
<td>90% (86-93)</td>
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<tr>
<td>2</td>
<td>179</td>
<td>5</td>
<td>77% (72, 83)</td>
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<td>3</td>
<td>89</td>
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<td>66% (59, 72)</td>
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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 leukaemia (ALL) (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 40% by ROC curve analysis. Among 95 patients who had available molecular data of both D+28 and 12 months, fifty nine (62.1%) patients had less than 40% of BCR-ABL1 transcript (VEMR) on Day+28. Long-term (n=26) patients achieved MMR at 12 months. However, only 27.8% (10 out of 36 patients) of patients without VEMR achieved expected survival (OS) & progression-free survival (PFS) rates for 24 months were 98.0% and 95.1% respectively. PFS rates by 24 months for VEMR and no VEMR group were 98.4% vs 88.8% respectively (p=0.04).

Table 1. Estimated OS in CP-CML patients receiving 3L treatment prior to ponatinib.

<table>
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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 on disease diagnosis and/or molecular relapse. Monitoring was performed when clinically appropriate.

Methods: Total BCR-ABL1 RTA 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 AmpliseqTM Designer Software were selected to cover the different mutational sites. Bar-coded libraries, constructed according to the AmpliSeqTM 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. 9 samples presented with one mutation: T315I (2), E255K (3), G250V (1), F359I (1), M237T(1) and E255A (1) and 9 harbourred combined 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: Advancements 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-α2B IN PATIENTS WITH SUBOPTIMAL MOLECULAR RESPONSE ON IMATINIB (NORDUTCHCML009)

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Background: The ability to “cure” a proportion of chronic myeloid leukemia patients (CML) makes treatment free remission (TFR) 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 suboptimal molecular response on imatinib (MR4.0), switching to nilotinib 300mg BD (Nil) PegIFN-α2b (Peg) 25 ug/week was introduced after 3 months, increased to 40 ug/week at month 6 if tolerable and continued until month 12. Adverse events were assessed according to CTCAE version 3. Flow cytometry assessments of T-, NK- and B-cell populations, acti-

E0108

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 are available and brought about faster and deeper clinical responses, 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 infarction (CI). 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 in this analysis. 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/BP) 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, Suitsa-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, 6 cases by nilotinib, 3 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, 7 high risk). The incidence rate of IHD and CI per 1000 person-years were 5.26 and 3.342 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 3.432 in the age-
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: 345 patients low risk, 307 intermediate risk and 129 high risk. Cutoff 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.

E1059
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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1University of Turin, Turin, Italy, 2The University of Texas MD Anderson Cancer Center, Houston, United States, 3Universitätsklinikum Jena, Jena, Germany, 4University of California San Francisco School of Medicine, San Francisco, 5Froedtert & the Medical College of Wisconsin, Milwaukee, 6Bristol-Myers Squibb, Princeton, 7Memorial Sloan Kettering Cancer Center, New York, United States

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) trending 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 response rates. The response rates were consistently higher for patients with ≥1 vs 0 comorbidities in both arms (MR4.5 on dasatinib: 46% vs 32%; MR4.5+ on 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 vs imatinib (36 or 35) vs imatinib (MR4.5: 42 or 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 rates 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 MYELOID LEUKEMIA PATIENTS WITH RESISTANCE OR INTOLERANCE TO BCR-ABL TKIS: 36 MONTHS UPDATE OF RADOTINIB PHASE 2 STUDY

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-ABL TKIs.

Aims: We update the long-term outcome of radotinib treatment in patients failed to either imatinib or dasatinib in clinical practice.

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 baseline, 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 (0-360.9) 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.

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.

Figure 1.
**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 70’s, 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 70’s, 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 from estimates of incidence rate of the disease by age and sex, population and demo-graphic projection from the National Institute of statistics and Economics 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 demo-graphic 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 80’s, 6 before the 2002, 17 in 2016, 22 in 2030 where the tendency infects, and 30 after 2040. Considering the 100% relative survival hypothesis, a target CML prevalence were nearly reached by 2050 to levels above 30 per 100,000 inhabitants. 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:** Our preliminary results suggested the involvement of miRNAs in CML-ABL levels regulation and in TKI response, supporting the search of a miRNAs TKI response profile that could predicts the response in CML patients. This information could act as powerful tool for the stratification of CML patients under Imatinib treatment showed higher levels of miR-451 associated with a higher reduction of BCR-ABL levels (lower than 0.01%) in CML patients and patients with higher BCR-ABL present lower levels of expression of miR-451. This miR was also down-regulated in LAMA-84, K562-RC and K562-RD comparing with sensitive cell line (K562); in K56-RD cells (created by discontinuous exposure), the degree of resistance is 18x. Statistical analysis was performed with ANOVA and multiple comparison tests, with significance levels of 95% (p<0.05).

**Results:** The miR-203 and miR-519c expression was not detected in any cell line or in CML patient. First, we correlated miRs expression with BCR-ABL levels. Higher levels of tumor suppressor miR-451 were associated with a higher reduction of BCR-ABL levels (lower than 0.01%) in CML patients and patients with higher BCR-ABL present lower levels of expression of miR-451. This miR was also down-regulated in LAMA-84, K562-RC and K562-RD comparing with sensitive cell line (K562); in K56-RD cells (created by discontinuous exposure), the degree of resistance is 18x. Statistical analysis was performed with ANOVA and multiple comparison tests, with significance levels of 95% (p<0.05).

**Methods:** To this end, we assessed the expression levels of miR-203, miR-21, miR-519c, miR-451, miR-26 and miR-16 (endogenous control) by TaqMan MicroRNA Assays in peripheral blood cells from 31 patients with CML at follow-up examinations. We also studied 4 CML cell lines, K562 a cell line sensitive to Imatinib, LAMA-84 a cell line with 4 copies of chromosome Philadelphia (Ph), and 2 Imatinib resistant cell lines models created in our lab (K562-RC and K562-RD). K562-RC cells, generated by continuous exposure to Imatinib, presented 8x times higher than the parental cell line (K562); in K56-RD cells (created by discontinuous exposure), the degree of resistance is 18x. Statistical analysis was performed with ANOVA and multiple comparison tests, with significance levels of 95% (p<0.05).

**Results:** The miR-203 and miR-519c expression was not detected in any cell line or in CML patient. First, we correlated miRs expression with BCR-ABL levels. Higher levels of tumor suppressor miR-451 were associated with a higher reduction of BCR-ABL levels (lower than 0.01%) in CML patients and patients with higher BCR-ABL present lower levels of expression of miR-451. This miR was also down-regulated in LAMA-84, K562-RC and K562-RD comparing with sensitive cell line (K562); in K56-RD cells (created by discontinuous exposure), the degree of resistance is 18x. Statistical analysis was performed with ANOVA and multiple comparison tests, with significance levels of 95% (p<0.05).

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(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 <1%), 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 for 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=2) 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 and 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).

Table 1.

<table>
<thead>
<tr>
<th>MDR protein</th>
<th>SNP</th>
<th>Genotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>C1236T</td>
<td>CT</td>
<td>32%</td>
</tr>
<tr>
<td>ABCB1</td>
<td>G2677T</td>
<td>GG</td>
<td>30%</td>
</tr>
<tr>
<td>ABCB1</td>
<td>G34A</td>
<td>AA</td>
<td>28%</td>
</tr>
<tr>
<td>ABCB1</td>
<td>C3435T</td>
<td>AA</td>
<td>22%</td>
</tr>
<tr>
<td>ABCG2</td>
<td>T129C</td>
<td>CT</td>
<td>32%</td>
</tr>
<tr>
<td>ABCG2</td>
<td>T29C</td>
<td>TT</td>
<td>52%</td>
</tr>
<tr>
<td>ABCG2</td>
<td>G241A</td>
<td>CC</td>
<td>32%</td>
</tr>
</tbody>
</table>

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.

THE INTRODUCTION OF SECOND-GENERATION TYROSINE KINASE INHIBITORS MAY REDUCE THE PROGNOSTIC IMPACT OF HIGH-RISK PATIENTS ASSESSED TO EUROPEAN TREATMENT AND OUTCOME STUDY (EUTOS) SCORE

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Background: The discovery of tyrosine kinase inhibitor (TKI) imatinib has revolutionized the conception 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 to 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, as these patients were able to be treated with 2nd TKIs. 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).

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 cooperation of participating in the observational study of European Leukemia Net (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.

<table>
<thead>
<tr>
<th>Number of patients diagnosed with CML during pregnancy</th>
<th>Patients treated with IM</th>
<th>Patients treated with HU</th>
<th>Patients treated with combination of IM and HU</th>
<th>Follow-up period</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>28</td>
<td>2</td>
<td>1</td>
<td>2.2 years</td>
</tr>
</tbody>
</table>

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 and pregnancy had the synchronistic onset of these events (table 1). Sokal low/intermediate/high and EUTOS low/high risk score was in 22/5/3 and 28/2 patients correspondingly. No data for risk score was in 1 patient. CML was diagnosed in 1st/2nd trimester in 18/16/6,66% correspondingly. Induced abortion was done before CML therapy was started in 10 (32%) around 10 obstetrician (week 6-17), spontaneous abortion happened in 1 woman (at week 8th).

Pregnancy was prolonged by consistent desire or religious reasons in 20 cases. Treatment of CML during pregnancy was initiated in 15 of 20 patients. Interferon alpha (IFN) was given to 5 women and 3 of them were switched to imatinib (IM) 400mg daily in 2nd-3rd trimester due to insufficient control of complete blood count. IM 400mg daily in 1st line was also taken by 9 women since 2nd-3rd trimester. In summary 12 women got IM from 2nd-3rd week (range 16-36). One woman got hydroxyurea (HU) from 3rd trimester till labor and 3 got HU shortly for 5-7 days before any other treatment in 1st-2nd trimester. Nineteen healthy newborns (including twins in 1 case) were born: 14 at term, 5 by preterm delivery at week 35-37. There were no birth abnormalities in 10 newborns exposed to IM in 2nd-3rd trimester. Two pregnancies under IM exposure are currently at week 17 and 31, developing normally. Four infants had low birth weight (<2500 g); 3 of them were exposed to IM and 1 for HU at pregnancy. Follow-up of children was uneventful with Me observation time 47 months (range 9-192) and Me observation time 38 months (range 9-63) for 10 infants exposed to IM in 2nd-3rd trimester. Twenty nine of 31 women with CML diagnosed at pregnancy are alive and continue TKI treatment, 2 women unfortunately died: 1 patient with postoned switch from IFN to IM during pregnancy progressed to blast crisis after labor and had further bone marrow transplant failure while 1 patient after induced abortion developed rapid blast crisis with BCR-ABL compound mutations including T315I.

Conclusion: The 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.

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 outcome, antitumor immunity by NK cells may contribute to the effects of TKIs. However, the response to TKIs depends on each case, and the determinants of it remain to be elucidated.

Aims: Given that NK cell function is regulated depending on the interaction between killer immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) class I molecules, we hypothesized that polymorphisms of KIR and HLA play important roles on the responses to TKIs. Then we performed allele genotyping of KIR and HLA with deep sequencing in CML patients, and here report our 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 copy/0.5 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, p value<0.05 was considered statistically significance.

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, 3.377 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 (HR 2.811, 95% CI, 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 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: To assess TKI discontinuation 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, patients were evaluated. Unrelated 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 (6 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, 1.0-10.2 months) and had undetectable levels of BCR-ABL1 transcripts 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/Conclusion: Our data demonstrated 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 BID OR IMATINIB


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, patients were evaluated. Unrelated 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.

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E1071 HYDROXYRUXEA SUPPRESSES BCR-ABL1 T315I+ CML CLONES IN VIVO AND IN VITRO AND SYNERGIZES WITH PONATINIB IN ELIMINATING TKI-RESISTANT CML CELLS

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Background: In chronic myeloid leukemia (CML), BCR-ABL1 T315I suppresses resistance to most BCR-ABL1 tyrosine kinase inhibitors (TKI). Long-term therapy with ponatinib, which suppresses BCR-ABL1 T315I, is problematic because of side effects. In addition, resistance against ponatinib may develop due to occurrence of compound mutations in BCR-ABL1. Therefore, alternative therapies to be considered. Hydroxyruxea (HU) has been used for (palliative) therapy in refractory leukemia and myelodysplastic malignancies. However, the effects of HU on TKI-resistant sub-clones have not been examined so far.

Aims: The aim of this study was to evaluate the effects of HU on CML sub-clones carrying BCR-ABL1 T315I mutations (isolated or in compound-configuration) in vitro and in vivo and to explore cooperative effects between HU and ponatinib.

Methods: Four BCR-ABL1 T315I+ CML patients were treated with HU (1-3 g/day) for 2 to 18 months. White blood counts (WBC), differential counts, and BCR-ABL1 transcript levels were reported. The BCR-ABL1 T315I+ CML BCR-ABL1 ratio determined by mutation-specific, ligation-dependent, PCR and next generation sequencing (NGS) was employed. To investigate the cell cycle effect of HU, BrdU (10 μM), KU812, KCL-22, and KCL-221+315I as well as Ba/F3 cells expressing BCR-ABL1 WT (Ba/F3p210WT), or mutant BCR-ABL1 (Ba/F3p210T315I) BCR-ABL1 WT or mutant BCR-ABL1 WT (Ba/F3p210T315I) were examined. Cell proliferation was quantified by 1H-tdTomato at a ratio of 1:1. Then, cells were exposed to HU, ponatinib, or...
Assessment of Treatment Outcomes in Patients on Tyrosine Kinase Inhibitor (TKI) Therapy

**Aim:** To further clarify the role of predictive biomarkers in molecular relapse after TKI discontinuation, we performed a retrospective analysis of patients who discontinued TKI treatment.

**Methods:**
- **Patients:** A total of 46 CML patients were included in the study (29 men; 17 women; median age, 58.5 years).
- **Methods:** Patients were continuously monitored for 18 months after TKI discontinuation. The ability to consistently detect low levels of BCR-ABL transcripts in clinical samples with initial BCR-ABL levels >10% was assessed.

**Results:**
- **Consistent results were observed in the both the diluted CML patient blood and spike-in CML cell lines or cell-line derived RNA studies for both the b3a2 and b2a2 transcripts, demonstrating an assay LoD of MR4.5 and below with a less than 2-fold difference at the LoD levels.**
- **With the clinical sensitivity study, eleven out of twelve low CML subjects were detected in at least 19 out of 20 replicates tested per subject over a range of 0.038% (SD=0.17 Log) to 0.0011% (SD=0.4 Log).** The overall ABL copy number present in clinical samples in each study was at least 5-10 times the required minimal ABL copy number of 232,000 to support a claim of MR4.5 and ≥100,000 for MR5.0.

**Summary/Conclusions:** The Xpert® BCR-ABL Ultra assay complies with the international guidelines for assay sensitivity achieving MR5 with 5-10 times more than the required ABL copies to confidently identify candidate patients that may benefit from the discontinuation of TKI therapy.

**E1073**

**XPERT® BCR-ABL ULTRA, A HIGH SENSITIVITY ASSAY WITH A LIMIT OF DETECTION REACHING MR4.5 AND BELOW ON AN INTERNATIONAL REPORTING SCALE**

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Background: The ability to consistently detect low levels of BCR-ABL transcripts in patients with chronic myeloid leukemia (CML) is important in the assessment of treatment outcomes in patients on tyrosine kinase inhibitor (TKI) therapy.

**Aim:** To further clarify the role of predictive biomarkers in molecular relapse after TKI discontinuation, we performed a retrospective analysis of patients who discontinued TKI treatment.

**Methods:**
- **Patients:** A total of 46 CML patients were included in the study (29 men; 17 women; median age, 58.5 years).
- **Methods:** Patients were continuously monitored for 18 months after TKI discontinuation. The ability to consistently detect low levels of BCR-ABL transcripts in clinical samples with initial BCR-ABL levels >10% was assessed.

**Results:**
- **Consistent results were observed in the both the diluted CML patient blood and spike-in CML cell lines or cell-line derived RNA studies for both the b3a2 and b2a2 transcripts, demonstrating an assay LoD of MR4.5 and below with a less than 2-fold difference at the LoD levels.**
- **With the clinical sensitivity study, eleven out of twelve low CML subjects were detected in at least 19 out of 20 replicates tested per subject over a range of 0.038% (SD=0.17 Log) to 0.0011% (SD=0.4 Log).** The overall ABL copy number present in clinical samples in each study was at least 5-10 times the required minimal ABL copy number of 232,000 to support a claim of MR4.5 and ≥100,000 for MR5.0.

**Summary/Conclusions:** The Xpert® BCR-ABL Ultra assay complies with the international guidelines for assay sensitivity achieving MR5 with 5-10 times more than the required ABL copies to confidently identify candidate patients that may benefit from the discontinuation of TKI therapy.


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Background: The ability to consistently detect low levels of BCR-ABL transcripts in patients with chronic myeloid leukemia (CML) is important in the assessment of treatment outcomes in patients on tyrosine kinase inhibitor (TKI) therapy. Particularly, BCR-ABL assays that are sensitive in the measurement of deep level response may aid in the identification of potential candidates for treatment discontinuation. Xpert® BCR-ABL Ultra detects the most common BCR-ABL transcripts below MR4.5 (Molecular Response at 4.5-log reduction) or 0.0032%, which is widely accepted as the clinical threshold that defines candidates who can safely discontinue TKI therapy.

**Aims:** The present studies were designed to verify the limit of detection (LoD) for the Xpert® BCR-ABL Ultra assay below MR4.5 on the International Scale (IS) clinical samples for both the b3a2 and b2a2 transcripts.

**Methods:** To overcome the challenge of testing numerous replicates requiring large volumes of patient samples, serial dilutions ranging from BCR-ABL/ABL levels of 10% to <0.001% (IS) were prepared as contrived samples using CML patient blood with initial BCR-ABL levels ≥10% (IS) and pooled blood from CML-negative patients, ranging from 10% to <0.001% (IS). Twenty-one replicate dilutions were measured for%BCR-ABL/ABL (IS). Determination of the LoD was performed by the statistical analysis to identify the lowest concentration of%BCR-ABL/ABL (IS) per test that can be reproducibly distinguished from negative samples with 95% confidence. The acceptable precision for%BCR-ABL/ABL (IS) is defined as the ability to detect at least a 3-fold difference for all concentrations tested.

In addition, analytical LoD studies were performed using spike-in CML cell lines and cell-line derived RNAs, carrying either b3a2 or b2a2 transcripts. Furthermore, the clinical sensitivity study was conducted using blood from twelve low BCR-ABL transcripts level CML patients on TKI therapy, who had achieved and maintained MMR (Major Molecular Response) [0.1% (IS)] with reporting below 0.05% (IS).

**Results:** Consistent results were observed in the both the diluted patient blood and spike-in CML cell lines or cell-line derived RNA studies for both the b3a2 and b2a2 transcripts, demonstrating an assay LoD of MR4.5 and below with a less than 2-fold difference at the LoD levels. With the clinical sensitivity study, eleven out of twelve low CML subjects were detected in at least 19 out of 20 replicates tested per subject over a range of 0.038% (SD=0.17 Log) to 0.0011% (SD=0.4 Log). The overall ABL copy number present in clinical samples in each study was at least 5-10 times the required minimal ABL copy number of 232,000 to support a claim of MR4.5 and ≥100,000 for MR5.0.

**Summary/Conclusions:** These LoD evaluations demonstrate that the Xpert® BCR-ABL Ultra assay complies with the international guidelines for assay sensitivity achieving MR5 with 5-10 times more than the required ABL copies to confidently identify candidate patients that may benefit from the discontinuation of TKI therapy.
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

Figure 1

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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 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 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 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 is required.

Aims: The recent report of conoidin A, as a specific Prx2 inhibitor, offers the possibility to perform cooxidation of cell systems, however, when its capacity is overwhelmed, the cell is exposed to oxidative stress, namely, when one enzyme is inhibited, LPO was highest for the pairs that included GPx. MBH was increased for all enzyme inhibitory conditions, when compared to the condition where all enzymes were active, and the lowest value was obtained when all enzymes were active, except for the case of conoidin 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 MBH, whereas, the lowest MBH was observed 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 the highest for the pairs that included GPx, MBH was increased for all enzyme inhibitory conditions, when compared to the condition with all enzymes active, except 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...
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 but these activities did not correlate with clinical severity. We found one patient (PK-4) was found with only one known missense mutation (R488Q), however we could not find any mutation in KLF1 of this patient suggesting that she might have other unidentified cis mutation involved gene regulation of PKLR. 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 splenohepatomegaly, 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).

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 haematology and oncology units for the first time were included if they had haematologic abnormalities (thrombocytopenia or anaemia) and/or anaemia, where other causes of splenomegaly has been excluded, are tested for GBA activity though a DBS sample. Only patients with DBS showing a GAU activity below normal values are recalled to confirm GBA enzyme deficiency using the gold standard GAU analysis in cell homogenate. For every tested patient clinical information are also collected.

Results: 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-7/1 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 5/25 patients (20%), with a prevalence of 9.9% (95% CI: 6.3-13.1%) 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 tool for GD in a selected population of children with splenomegaly and/or thrombocytopenia considered at increased risk for the disease. The use of a 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.

E1079
CIRCULATING MICROPARTICLES IN CONGENITAL AND ACQUIRED HAEMOLYTIC ANAEMIA
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Background: Microparticles (MPs) are small particles budding from cells, which contain variable amounts of proteins, miRNA and cytosol 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 haemolytic conditions, both congenital and acquired. Elevated levels of circulating MPs have been described in several haemolytic conditions, including sickle cell anaemia, thrombocytopenia, haemolytic uraemic syndrome, and thrombotic thrombocytopenic purpura.

Aims: To evaluate platelet MPs (PMP), tissue factor expressing MPs (TF MPs), endothelial MPs (EMPs) and microparticles expressing single antigens (CD41, CD142 or CD142) levels in in other haemolytic anaemias, such as hereditary spherocytosis (HS), elliptocytosis (HE), stomatocytosis (HSt), red cell enzymatic defects, congenital dyserythropoietic anaemia (CDA), autoimmune haemolytic 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 annexin V-APC, CD41-FITC, CD142-PE and CD144 PerCP-Cy5.5 in 5 Hepes buffer in the presence of 15 mM CaCl2 and 1 μM of r-Hirudin for 30 min. Samples were diluted with 500 μl Annexin Binding Buffer. 25 μl of fluorescent iron deficient CD41+ MPs 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 number of MPs in controls (n=30) was 8.3±5.5 (range 2.2-28.7); 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 patient groups.

Discussion: MPs levels were abnormal in both congenital and acquired haemolytic conditions. MPs levels correlate with the degree of anaemia and haemolysis and with the duration of disease.

Summary/Conclusions: These preliminary results suggest that MPs levels are abnormal in both congenital and acquired haemolytic 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
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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 laboratory criteria, age, gender, diagnostic group and medical treatment 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 elderly 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 (33.1%), 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/or thrombocytopenia considered at increased risk for the disease. The use of an appropriate 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.

Summary/Conclusions: 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.

E1079
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.
**E1080**

PIEZ01 MECHANOTRANSDUCTIVE PROTEIN MUTATIONS IN RBCS: WHEN THE PHENOTYPE IS BEYOND HYEMOLOGIC 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 hemolytic anemia 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 highly 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 FAM38A 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 as 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 xerocytes in PBS. 13/26 patients had reticulocytosis, a median reticulocyte count of 101 x109/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 both. Only 5 patients presented with anaemia (Hb <12g/dL), 2 macrocytic and 3 normocytic. One patient was not anaemic but he also had a hemolytic anaemia 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 xerocytes 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 xerocytes 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 pathological pattern is unusual and the diagnosis that is presented 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 lead to severe chronic anemia, iron overload, and splenomegaly. 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 (coPKR).

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 previously delivered PKD-hematopoietic 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 coRPK 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 RPK impaired. The decrease of PK activity validates this approach as a human model for PKD.

**E1082**

PHYSIOPATHOLOGY OF HEREDITARY XEROCYTOSIS : PIEZO1 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 ektacytometry and EMA assay. PIEZO1 and KCNN4 coding regions were analyzed by Sanger sequencing in all HX patients. Hemoglobin oxygen affinity was evaluated using p50 measured on venous blood on a Hemoxanalyzer or a Radiometer blood gas analyzer. 2.3 diphosphoglycerate (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 PIEZO1, no gene variation was identified in KCNN4. 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 27 for which biochemical study showed a high likelihood of pathogenicity. For all HX patients, p50 values were under the normal range (median 21.1, range 19.7-23.4, normal range 25-29 mmHg), contrasting with HS patients 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 PIEZO1 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 we observed. In particular, we showed a high tissue hypoxia and consequently a high reticulocytosis, providing a clue to explain the compensated hemolysis frequently observed. However, the links between PIEZO1 mutations, reduced cell enzymatic activity and erythropoiesis need to be clarified and a proteomic and a metabolomic approach is under investigation. Of interest is the clinical point of view, HX diagnosis is sometimes difficult and this low p50 value, easily measured on venous blood, represents a useful new diagnosis tool for HX.
Background: Viral infections, most commonly by cytomegalovirus (CMV), Epstein-Barr virus (EBV), polyoma virus 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 multi-pathogen-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 1.5x10^7 mononuclear cells with viral (CMV, IE1, pp65; EBV: EBNA1, LMP2, BZLF1; BK: Large T, VP1) and Asp pepmixes (Crf1, Gel1, SHMT) and culturing for 10 days. The specificity of mp-STs was analyzed by IFN-γ Elispot. A total of 1.5x10^5 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. NK-92MIhCD16 cells were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assayed by histology and immunohistochemistry.

Results: We generated 2.3±5x10^7 cells mp-STs (12-fold expansion). All cell lines were polyclonal expressing cytokine receptor immunomarkers and specific against Asp [spot forming cells (SFC)/2x10^5cells: 31±52] and the targeted viruses, if derived from seropositive donors [SFC/2x10^5cells: CMV, 63.7±267; EBV: 744±158; BK: 578±118]. To first assess the safety issue, we asked whether mp-STs can induce acute graft-versus-host disease (aGvHD) in myelo-ablated mice. While DLI mice succumbed early and histologically confirmed aGvHD and succumbed by day 20, mp-ST-mice survived free of aGvHD until the day of sacrifice (d28). To assay 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 (d28). While 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 "one 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.

E1085

GENE-MODIFIED NK-92MI CELLS EXPRESSING A CHIMERIC CD16/CD64-BZLF1 BB-Z RECEPTOR EXHIBIT ENHANCED CANCER-KILLING ABILITY IN COMBINATION WITH THERAPEUTIC ANTIBODY

A. Schematic representation of the CD16-BB-ζ and CD64-BB-ζ FcγRIII 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.

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-92 mi 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 CD3a 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, 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 PHARMACOLOGY ACTIVITY OF BISPECIFIC ANTIBODIES IN HEMATOLOGICAL SAMPLES


Background: The PharmaFlow automated flow platform has achieved 85% clinical correlation with AML samples with its novel Native Environment assay (NEA). Recently, novel B-specific antibodies (BsAbs) or analogous constructions acting through the formation of an immunologic synapse between T-cells (CD3) and a tumor-associated surface antigen (TAA) have been used as immunotherapy leading to T-cell activation and serial lysis of tumor cells. We have developed an automated flow cytometry assay for bispecific antibody screening that keeps intact both basal effector to target (E:T) ratios and Native environment using whole blood or bone marrow samples. In this context, the PharmaFlow platform selects different in vitro T-cytotoxicity effects across patients identifying best patient candidates for adoptive antitumor immunotherapy with BsAbs. The integration of Effective E:T ratios and pharmacological parameters better predict the in vivo response of BsAbs. Because of the high capacity of the PharmaFlow platform, additional antibodies constructions alone or in combinations with immunomodulatory agents could be tested to identify the best agents or immunotherapeutics combinations in hematological diseases.

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 CD123xCD3 (Creative Biologics) and 7 CLL and 3 B-ALL samples with Blinatumomab (Amgen). When appropriate, basal quantification of TAA was performed by flow cytometry (FCM). A flow platform named PharmaFlow by FCM analysis was used to count by FCM how many tumor cells were killed by every activated T-cells, here called effective E:T ratio. For each sample, 8-colour FCM staining was performed to simultaneously analyze the leukemic population, activated CD4 and CD8 T-cells and the residual normal cells. EC50 or Emax was calculated to evaluate potency or efficacy. Kinetics of activity was measured repeating the dose response curves in 3 different days.

Results: Most of the samples present both T-cell activation (CD25+) and an effective lysis of tumor cells after BsAbs exposure in a time and dose dependent manner (Figure 1), even starting with low basal E:T ratios (<1:100). For AML, basal quantification of CD123 by FCM density does not reflect a correlation with the in vitro response, therefore differences in T-cell cytotoxicity or leukemic immunoresistance were observed between samples in terms of EC50 or Emax even more marked between CLL samples. The integration of effective E:T ratios, EC50, Emax, and kinetics allow us to generate an in vitro response model and select those samples with higher T-cell cytotoxicity after the different BsAbs exposure. Interestingly, many of the samples for all the BsAbs leave a significant proportion of live cells, even at the higher BsAbs concentrations or with a remarkable expansion of activated T-cells that suggest the use of immunomodulators to unlock this immunoresistant status.

Summary/Conclusions: We have developed an automated flow cytometry assay for bispecific antibodies screening that keeps intact basal effector to target (E:T) ratios and Native environment using whole blood or bone marrow samples. In this context, the PharmaFlow platform selects different in vitro T-cytotoxicity across patients identifying best patient candidates for adoptive antitumor immunotherapy with BsAbs. The integration of Effective E:T ratios and pharmacological parameters better predict the in vivo response of BsAbs. Because of the high capacity of the PharmaFlow platform, additional antibodies constructions alone or in combinations with immunomodulatory agents could be tested to identify the best agents or immunotherapeutics combinations in hematological diseases.

E1087
HUMANIZED CD7 NANOBODY-BASED IMMUNOTOXINS EXHIBIT PROMISING ANTI-T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA POTENTIAL

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Background: The key to translating those immunotoxins into clinics, we humanized the nanobody sequences (designated as dhuVHH), 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, dVH6-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 bind specifically to CD7-positive T lymphoma cell lines (designated to CD7-negative control cells. Laser scanning confocal microscopy found that these proteins can be endocytosed into the cytoplasm after binding with CD7-positive cells, and that this phenomenon was not observed in CD7-negative cells. Western blot experiments showed that all immunotoxins retained the highly effective and specific growth inhibition activity in CD7-positive leukemia cell lines 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 models 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 disappointing.
SUMMARY/CONCLUSIONS: We have successfully constructed a targeted CD7 molecule modified nanobody (CD7 molecule improved nanobody) immunotoxin dhuVH6-HPE38 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 by the CB-NK MM 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 cytototoxicity 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 cytototoxicity against MM cells. Furthermore, pretreatment of MM cells with Fluvastatin (3 μM) increased CB-NK cytototoxicity 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 generation of CAR-expressing MM cells cell-free MM cell culture. Furthermore, FACs sorting experiments showed that MM cells exposed 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 efficiency of CAR immunotherapy against BCMA, suggesting the potential of statins as an adjuvant in CAR-NK immunotherapy against MM.

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 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, four treatment groups were designed to be a mimicking 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 vitro 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 (MDSCs), 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.

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 bar-coded (MBC) adapters and gene-specific primers (GSPs), enabling NGS-based immune chain mRNA interrogation from a single amplicon. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating clonal dropout due to some over or under amplification.

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 by PCR amplifying DNA 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, Immunovarse™ (IGH for B-cell and T-cell Ig), TCR for B-cell and T-cell, 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 mimicking protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) Pom + Dex, and 4) DCs + Pom + Dex. After vaccination, preclinical and in vitro immunological responses were evaluated.

Results: Among four treatment groups, DC combined with POM and DECA 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 (Tregs) 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.

Summary/Conclusions: This protocol combined with POM and DECA synergistically enhance the anti-tumor immunity in a murine myeloma model, by skewing immuno-suppressive status toward immuno-suppressive status in tumor microenvironment.
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 10^5 cells per flask, and then 10^6 allogeneic lymphocytes from one donor were added to all MSCs cultures. For lymphocytes activation 5ng/ml phytomagnesium (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 expression of CM and PD-1+ CD4+ cells was measured by flow cytometry. Activation markers CD25, CD38, CD69, HLA-DR expression of CM and PD-1+ CD4+ cells was lower, compared to group A and control samples. At the same time the number of effector T-cells were analyzed on 4th day of cultivation. p<0.05 was considered statistically significant; all data are presented as medium ± SEM.

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 (R^2=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 naive cells compared to control (47.4±3.5% and 54.9±2.0% for group A and B vs 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 MSC 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.

The materials are supported by grant from the Russian Science Foundation, Project № 16-15-00102.

GRANULOCYTE COLONY STIMULATING FACTOR AND ERYTHROPOIETIN ENTIRELY GIVEN FOR NEONATES RECOVERING FROM GUT SURGERIES: RANDOMIZED CONTROLLED TRIAL

E.1093

Feeding intolerance is a common problem among neonates recovering from surgery for congenital abnormalities of the gastrointestinal tract (GIT) such as small bowel atresia, omphalocoele 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 fasting 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 oro-gastric or nasogastric administration of 20 mL/kg/day of sterile isotonic solution that contained cytokine concentrations comparable to what they would have ingested from amniotic fluid in utero.

Aims: 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 in an amount included within similar uptake with suckling

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 Enterally; 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 and higher rate of weight gain (p<0.05). No significant increase was found in the level of WBCs count, hemoglobin and hematocrit values either pre-initiation or 7 days after administration of SAFE (p>0.05).

Summary/Conclusions: This study provides further insights on the importance of neonatal outcomes and help to decrease morbidities from post-operative malnutrition and feeding intolerance. Enteral administration of rhG-CSF and rhEPO may play a critical role in preventing viillos atrophy, thereby, reducing feeding intolerance in neonates recovering from surgeries for congenital bowel abnormalities.
Results: Although a high toxicity and low efficiency were observed with the elecrotransfection 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. More recently, site-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 REFRACTORY ACUTE MYELOID LEUKEMIA Z. Ernarah1,2, S. Shaama1,2, M. El-Zaafarany1,2, N. Essa3,4, M. Khalaf1, 1Medical Oncology Unit, Oncology Center, Mansoura University, 2Medical Oncology Unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, 3Clinical Hematology Unit, Oncology Center, Mansoura University, 4Clinical Hematology Unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, Mansoura, 2Clinical Hematology Department, Hematology and Oncology Hospital, Maadi Armed Forces Medical Compound, Cairo, Egypt

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.

Aims: To analyze the safety and efficacy of non-engraftment haploidentical cellular therapy for patients with refractory acute myeloid leukemia by assessment of bone marrow 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, cytarabine 1gm/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 25 days (range, 13-40) days, while the other five patient did not. Two patients attain platelet recovery with median time 34.5 (range, 29-40) days, while the other five did not. The cellular therapy did not elicit statistically significant changes in bone marrow blast% over time, F(2, 10)=1.558, p=.258, partial η2=.256, 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.

E1096 ALTERATIONS IN T-CELLS 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 always effective. 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 naïve 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 allogeneic 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 IFNγ (gMSCs). Activation markers CD25, CD38, CD69, HLA-DR and CD2 were measured using flow cytometry as well as distribution of naïve (CD45RA+CD62L+) and effector memory T-cells (CD45RA+CD62L-) in 1st, 2nd, 3rd and 4th day of co-culture.

Results: By the fourth day of incubation the proportion of naïve CD4+ cells 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 naïve T-lymphocytes transit into effector cells. The proportion of CD4+PD-1+ T-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 gMSCs did not change (p=0.0125).

The proportion of HLA-DR+ both on CD4+ and CD8+ cells in 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+ (6.2±0.3% to 15.6±1.1%, p=0.005) and CD8+/HLA-DR+ (9.7±0.8% to 26.0±3.7%, p=0.024). So allogeneic MSCs 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 4th day. MSCs inhibited this increase - the proportion of CD4+ central memory cells increased from 24.4±2.7% to 46.2±5.4% (p=0.047). Thus the interaction of T lymphocytes with PHA-lymphocytes with MSCs inhibited their activation and preserved naïve state.

Summary/Conclusions: The composition of lymphocyte population changes during cultivation. The proportion of naïve cells reduced, while the number of effector and the proportion of PO-1+ increased, indicating the lymphocyte activation probably due to the presence of xenogeneic 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 lymphocytes activation. It was shown that MSCs both on naïve and effector lymphocyte condition have an inhibitory effect on their activation. The materials are supported by grant from the Russian Science Foundation, Project № 16-15-00102

E1097 OPTIMIZATION OF TRANSDUCTION CONDITIONS WITH GMP LIKE LENTIVIRAL VECTORS FOR THE GENE THERAPY OF PYRUVATE KINASE DEFICIENCY

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Background: Pyruvate kinase deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene. PKD is the most common erythroid inherited enzymatic defect causing chronic nonspherocytic hemolytic anemia. PKD is associated with reticulocytosis, splenomegaly and hepatic iron overload, and may be life-threatening in severely affected patients. To-date, allogeneic bone marrow transplant remains the only curative treatment for severely affected patients but has been employed infrequently. Splenectomy confers reduced transfusion-dependence in many patients, but 10-15% of PKD patients remain transfusion-dependence despite splenectomy, which confers increased likelihood of transfusion reactions. Precipitation of the PKD genes that are conducted in pyruvate kinase deficient mice have shown the safety and the efficacy of a new PCoRPKW-17 therapeutic lentiviral vector that has been granted orphan drug designation (EMA: EU/3/14/1330; FDA: DRU-2016-5168).

The aim of the study was to determine the distribution of naïve and effector cells in lymphocytes co-cultured with MSCs.
Aims: In order to develop a gene therapy clinical trial for PKD we are optimizing transduction procedures compatible with a clinical application.

Methods: Using a GMP-grade lentiviral vector production according to manufacturing processes of the CMO 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 compared with different viral concentration and transduction conditions.

Results: Increased doses of virus concentration revealed, as expected, increasing levels of transduction that ranged 40-90% both by scoring transduced colony forming units and by flow cytometry analysis in hematopoietic progenitors maintained for 15 days in liquid culture. Analysis of vector copy number (VCM) by qPCR ranged from 0.5 to 3 VCM/cell, demonstrating good transduction efficiency, compatible with a clinical application. Two cycles of transduction showed an increased level of transduction at limiting concentrations of the viral vector, improving the VCM 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 PROPERTIES

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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 (IFNg) 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-genic 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 IFN-g 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-Lymphocytes). For each of the MSCs cultures the mean fluorescent signal intensity (MFI) of CD90 PE, CD54 APC, HLA-DR APC was measured. Relative expression level (REL) of CD90, CFH, PTGES, IL6, CSF1, ICAM-1 was analyzed in gMSCs by RT-PCR. MFI and REL were investigated on the 1st, 2nd, 3rd and 4th days of cultivation.

Results: IFN-g transduction showed an increased level of transduction at limiting concentrations of the viral vector, improving the VCM up to 2-fold. Increased doses of virus concentration revealed, as expected, increasing levels of transduction that ranged 40-90% both by scoring transduced colony forming units and by flow cytometry analysis in hematopoietic progenitors maintained for 15 days in liquid culture. Analysis of vector copy number (VCM) by qPCR ranged from 0.5 to 3 VCM/cell, demonstrating good transduction efficiency, compatible with a clinical application. Two cycles of transduction showed an increased level of transduction at limiting concentrations of the viral vector, improving the VCM 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.

E1099

FUNCTIONALy HETEROGENEOus POPULATION OF MEGAKaryocyte Progenitors cEASES Abruptly bY E10.25 and constITUTES a FUNCTIONALLY DISTINCT POPULATION

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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 efficiently 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).

Hematopoiesis, stem cells and microenvironment

E1100

C-TYPE LECTIN-LIKE RECEPTOR 2 SPECIFIES A FUNCTIONALLY DISTINCT SUBPOPULATION OF MEGAKARYOCYTE-BIASED 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 efficiently 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).

The materials, supported by grant from the Russian Science Foundation, Project № 16-15-00102.
Aims: In this study, we analyzed in vivo dynamics of CLEC2high HSCs to clarify their functional roles in adult hematopoiesis.

Methods: In this experiment, we defined Lin–Sca1+Kit+CD150+CD34+ cells as HSCs and Lin–Sca1+Kit+CD150+CD41+ as MPPs. We performed transplantation assays using HSCs isolated from EGFP transgenic (CAG-EGFP) mice to trace donor-derived HSCs and their progeny. Excepting enucleated transplantation in non-leukemic and GFP– mice, CLEC2high and CLEC2low HSCs were transplanted into lethally irradiated mice, respectively. Chimerism and lineage distribution of donor-derived cells were evaluated periodically by tracing EGFP. Secondary transplantation was performed by transferring 1x10^7 BM cells from the recipient mice 16 weeks after the first transplantation.

Results: Bone marrow arrest revealed that both EGFP+CLEC2high and CLEC2low donor-derived HSC populations were detected for up to 12 weeks after transplantation. Also, these subsets were capable of generating all lineages of cells transplanted mice. Interestingly, CLEC2high HSCs generated CLEC2high megakaryocytes (MKs), whereas CLEC2low MKs showed only steady output of MKPs and MKPs, in contrast with CLEC2high MKs. Consistent with these reciprocal transplants, both types of HSCs could have reconstituted hematopoiesis in the secondary recipients. However, CLEC2high HSCs showed significantly reduced repopulating activity than CLEC2low cells, especially at 12 weeks after transplantation (mean of EGFP+HSC proportion in the primary recipients with CLEC2high HSCs vs CLEC2low HSCs, each = 5%): 21.1% ± 6.6% at 4 weeks (p = 0.054); 2.14% ± 48.3% at 12 weeks (p = 0.05). In addition, the recipient mice transplanted with CLEC2low HSCs kept high chimeric levels of EGFP+CMP and MEP, while these levels decreased in the recipients with CLEC2high HSCs. On the other hand, CLEC2high HSCs yielded 2.8-fold more MKPs than CLEC2low HSCs in short-term grafts (1 to 2 weeks after transplantation) (p < 0.05). Consistent with this finding, CLEC2high HSCs yielded more CD4+ platelets than CLEC2low HSCs by 6.0-fold at 1 week after transplantation (p < 0.05), which peaked 10 weeks earlier than in CLEC2low recipient mice. These platelets yielded through the transplantation of CLEC2high MKs were rare at several levels during reconstitution. Furthermore, treatment with fostamatinib (R788), a Syk kinase inhibitor that is an indispensable component for CLEC2 signaling, blocked more potent and rapid megakaryopoesis in the CLEC2high recipients, indicating that CLEC2 signaling is essential for rapid and enhanced megakaryopoiesis from CLEC2high HSCs.

Summary/Conclusions: Here, we showed that CLEC2 expression on HSCs demonstrates its oscillation for serving as a potent source of megakaryopoesis, and found that CLEC2/Syk signaling would be involved in differential regulation between CLEC2high and CLEC2low HSC subtypes.

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 hematological chimerism with no residual or recurrent leukemia, but a hypo- or aplastic bone marrow (BM) with 2 or 3 of the following: (1) neutrophils ≤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 factors for PGF, pre-HSCT risk factors with a poor graft function (PGF) post-HSCT. All in training group and validation group pre-HSCT, significantly reduced percentage of BM EPCs were observed in PGF patients than those in GGF patients, whereas no significant differences were found in the percentage of BM HSCs and their progeny. Persistently low percentage and high levels of ROS in post-transplant patients with PGF, and GGF groups, significantly lower percentages of BM EPCs and HSCs, whereas remarkably higher ROS levels were observed in BM EPCs and HSCs in PGF patients.

Methods: To compare the reconstitution kinetics of BM EPCs, HSCs and their functional roles in adult hematopoiesis. In this regard, we previously reported that CLEC2/Syk signaling would be involved in differential regulation between CLEC2high and CLEC2low HSC subtypes.

Summary/Conclusions: Here, we showed that CLEC2 expression on HSCs demonstrates its oscillation for serving as a potent source of megakaryopoesis, and found that CLEC2/Syk signaling would be involved in differential regulation between CLEC2high and CLEC2low HSC subtypes.

E1102

EFFICIENT LYMPHOID DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS REQUIRES CXCR4 DESENSITIZATION

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Background: The Warburg Hypogammaglobulinemia Infections and Myelokathexis Syndrome (WS) is a rare immunohaematological disorder characterised by chronic lymphopenia. It is mostly caused by inherited heterozygous autosomal gain-of-function mutations in CXCR4, which engender a distinct truncation in the C-terminal domain and lead to a desensitization-resistant receptor. Given that CXCR4 is widely expressed on non-hematopoietic cells and virtually on all leukocytes at multiple stages of development, one possibility could be that the WS-associated peripheral blood lymphopenia is a consequence of skewed production, differentiation or distribution of lymphocytes related to altered CXCR4-mediated signaling. Recently, we have been able to replicate the hematologic phenotype of WS using a knock-in mouse strain that harbors the WS-linked heterozygous CXCR4S338X mutation causing a distal truncation of the last 15 residues of the C-terminal domain (Balabanian et al., Blood, 2012). Mutant mice displayed lymphopenia with enhanced migration to Cxcr4+ tissues and failed to maintain antibody titers after immunization (Biajoux et al., Cell Reports, 2016). First-line analyses of +/-103 mice suggested developmental defects at the pre/pre-B cell stage in the bone marrow (BM) and during the early double-negative stages of thymocyte maturation. However, whether impaired lymphopoiesis stems from an upstream cell-intrinsic or cell-extrinsic hematopoietic defect remains to be established.

Aims: We took advantage of our relevant knock-in model and the access to blood samples from WS patients to investigate the impact of CXCR4 desensitization on BM and extra-medullary splenic hematopoiesis and recirculation of hematopoietic cells (HPCs).

Methods: The global hematopoietic development, including quiescence, cycling and survival properties of HSCs, was examined in non-manipulated and BM chimeric mice using flow-cytometric and clonogenic-based assays. Cxcr4 expression and function were assessed using internalization, in vivo homing and AMD3100-promoted mobilization experiments. Both multipotency and self-renewal abilities of HSCs have been assessed using serial BM transplantation experiments. Immune-phenotypic and clonogenic analyses of HSPCs were performed from blood samples of five WS patients and age-, sex-matched healthy control donors.

Results: We showed that Cxcr4 desensitization is required for quiescence/cycling balance of murine short-term HSCs and their differentiation into multipotent (MPPs) and downstream lymphoid-biased progenitors (i.e. LMPPs and CLPs). Alteration in this negative feedback mechanism resulted in dramatic decrease of circulating HSPCs in five patients with WS. This was also evident in WS mice and mirrored by accumulation of HSPCs in the spleen, where enhanced extramedullary hematopoiesis occurred.

Summary/Conclusions: Efficient Cxcr4 desensitization is critical for the lymphopoietic differentiation of HPCs and its impairment is a key mechanism underpinning the lymphopenia observed in mice and likely in WS patients.
Background: GATA4 is a transcription factor expressed in mesoderm and endoderm during development. Members of the family such as GATA-1-3, 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 G2Cre;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 Yap 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 around 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 adult mice and contributed in the same proportion to the lymphoid, myeloid and erythroid lineages. Gata4 expression decreased with age in the G2Cre driver is lethal by midgestation (Delgado et al., Hepatology, 2014, 59:2358). The anemia observed in the G2Cre;Gata4floxed/floxed embryos was positive for leukocyte and megakaryocyte markers, suggesting that a lineage of hematopoietic cells could derive from GATA4 expressing progenitors.

Figure 1.

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 extraembryonic (placental) origin. Both lineages basically express lymphoid, myeloid and erythroid genes (HBB, HBG1, and SLC4A1), whereas repressed that of HBA, HBG2, and SLC4A1. We demonstrated that activating marks (H3K4ac, H3K9ac, and H3K4me3) were significantly increased at the GATA1-activated gene loci, whereas repressed that of GATA1-activated genes. Therefore, we conclude that in hematopoietic stem cells, GATA4 expression is required to maintain HSCs in a progenitor state.
E1106
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 the multitude of 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 DNAp 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 (CFD43+ and CD133+). Global DNAp profiles were generated with the Infinium HumanMethylation450 BeadChip platform and gene expression profiles with the Human Affymetrix GeneChip® U133 version 2.0 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 DNAp patterns: several CpG sites revealed significant differences in DNAp amounts upon knockdown of Tr.2 and Tr.1+3 (8,905 and 352 CpGs, respectively; n=3, adjusted p-value <0.05). Notably, these patterns were regulated in the opposite direction upon overexpression of the same transcripts. Knockdown of Tr.4, which does not have the DNA-methyltransferase domain, did not evoke significant changes in DNAp. Furthermore, modulation of DNMT3A splice variants resulted in transcript-specific gene expression changes, which may at least partly be attributed to the DNAp changes.
Summary/Conclusions: Our results demonstrate that the various splice variants of DNMT3A have different functional sequel on HSPCs. Knockdown and overexpression resulted in opposite and transcript-specific DNAp changes. Thus, alternative splicing of DNMT3A is relevant for site-specific epigenetic modifications in hematopoietic development.
E1100

BONE MARROW MYELOPOIESIS INDEPENDENTLY OF CANONICAL NOTCH SIGNALLING

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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 (Rbpfj). 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), erythroid (E) and granulocyte-macrophage (GM) 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 pathways distinct from canonical Notch signaling.

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 was 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 of CFU, and transplantation experiments for the demonstration of functional clonogenicities.

Summary/Conclusions: Our data suggest that HSCs can be further subdivided into subfractions based on their CD35 expression. CD35+ cells may have a functional advantage in that they may be able 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.

E1111

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−, CD90+, and Lin−. This approach is based on the assumption that HSCs reside in functionally heterogeneous subpopulations, including multi-potent and/or lineage-agersensitive progenitors (Notta:2016hi) 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 surface marker(s) that enables prospective isolation of functionally-distinct HSC subpopulations.

Methods: We produced a PMMA-based 3D scaffold and compared the growth of CML and AML cell lines grown in this scaffold in the presence and absence of imatinib and doxorubicin respectively.

Results: We produced a PMMA-based 3D scaffold and compared the growth of CML and AML cell lines grown in this scaffold in the presence and absence of imatinib and doxorubicin respectively.

Summary/Conclusions: Our data suggest that HSCs can be further subdivided into subfractions based on their CD35 expression. CD35+ cells may have a functional advantage in that they may be able 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.

Methods: Scaffold production: PMMA solution was prepared by dissolving PMMA in chloroform and adding appropriate amount of hydroxyapatite to poly-MMA in chloroform. 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 was 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 of CFU, and transplantation experiments for the demonstration of functional clonogenicities.

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Methods: Scaffold production: PMMA solution was prepared by dissolving PMMA in chloroform and adding appropriate amount of hydroxyapatite to poly-MMA in chloroform. 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 was 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 of CFU, and transplantation experiments for the demonstration of functional clonogenicities.

Summary/Conclusions: Our data suggest that HSCs can be further subdivided into subfractions based on their CD35 expression. CD35+ cells may have a functional advantage in that they may be able 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.

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fibre. 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 cells HS-5 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 HAEMATOLOGICAL MALIGNANCY AFTER HEMATOPOIETIC TRANSPLANTATION

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Background: The leukemic transformation of otherwise healthy donor stem cells provides a useful model for the study of leukemogenesis. The leukemic transformation of otherwise healthy donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT 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.

Methods: Case 1: A 43-year-old female diagnosed with lymphoblastic leukemia (B-ALL) at diagnosis and in whom whole-exome sequencing (WES) was performed in bone marrow (BM) samples from recipient at different times after allogeneic hematopoietic 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). Case 2: A 65-year-old male diagnosed with mantle cell lymphoma, who developed MDS 45,XX,-7,del(12)(p12) 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, 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, 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, 16 months after unrelated cord blood transplantation (UCBT).

Results: Donor cell leukemia (DCL) and myelodysplastic syndrome (DC-MDS) developed in 45,XX,-7,del(12)(p12) of donor origin, 16 months after unrelated cord blood transplantation (UCBT). 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 cells HS-5 to the scaffold reduced even further the sensitivity of K562 or HL60 to imatinib or doxorubicin, respectively.

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**E1115**

**POTENTIAL PREDISPENSING 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 SFB31, are mutated in both myeloid and lymphoid malignancies. This could indicate a possible genetic link between myeloid and lymphoid malignacies. 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 myeloproliferative 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, immunohistochesmistry, cytogenetics, and flowcytometry analysis in accordance to the WHO classification. For each patient mononuclear cells (MNCs) from blood or bone marrow were isolated using Ficoc gradient 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 CLC Biomedical Genomics Workbench (Qiagen) m_clc 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, D1309*4), SMARCA1 (2114C>T, T705I), HEGQ (393_397delAGGTG, 1323+16), SWI5 (652C>T, R218*), LIG1(2168A>G, Q761R) and PRKD2(502G>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(44delIC, P159fs*2). Furthermore, we identified the somatic mutational landscapes of the malignant clones using T-cells as germline tissue for the patients’ diagnoses, indicating development of two independent malignancies.

**Summary/Conclusions:** Our results suggest a possible role of germline variations in the susceptibility to development of concomitant de novo hematological cancers as well as t-AML. However, further studies including more patients are needed to confirm this hypothesis.

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**E1116**

**THE MUTATIONAL LANDSCAPE OF DNMT3A MUTATIONS IN CLONAL HAEMATOPOIESIS OF INDETERMINATE POTENTIAL. CHIPPING AWAY AT THE PROBLEM**

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**Background:** Dysfunction of epigenetic modifiers contributes significantly to the pathogenesis of acute myeloid leukaemia (AML). One frequently mutated gene involved in epigenetic modification is DNMT3A (DNA methyltransferase-3-alpha). Approximately 22% of de-novo AML and 36% of cytogenetically normal AML, are found to have DNMT3A mutations and around 60% of these mutations affect the R882 codon. In particular, the R882H mutation has been associated with a poor prognosis and survival outcomes for patients. A large number of DNMT3A mutations are present in clonal cells in healthy individuals with no characteristics of haematological malignancy and is termed as clonal haematopoiesis of indeterminate potential (CHIP).

**Figure 1.**

**Aims:** We aimed to compare here the locations and types of mutations identified in AML and in CHIP in the DNMT3A gene by several different studies.

**Methods:** To review the mutations found in CHIP and AML, we carried out an extensive literature search of CHIP studies and AML studies that had mapped a large number of mutations in this gene. Mutations were collated to form several diagrams illustrating and comparing these findings.

**Results:** When DNMT3A mutations in CHIP were compared to mutations in AML the R882 residue was still found to be the most frequently mutated residue in both CHIP and AML. Figure 1 clearly illustrates the mutations in comparison to AML. However, only 13% of all reported mutations were found at the R882 residue in CHIP, while in AML 60% DNMT3A mutations are found at the R882H mutations.

**Summary/Conclusions:** Analysis of the mutational landscape of CHIP has clearly highlighted the role of DNMT3A mutations in clonal haematopoiesis in older healthy individuals, the significance of such preleukaemic clones is yet to be determined.

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**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 hematology 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 hematology 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 hematology 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.
Hodgkin lymphoma - Clinical

BASLINE 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 (rr) 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 rr 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 rr 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 rr 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 (≥ 7.78x10³/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 ≥ 7.78x10³/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): NA) whereas 5 out of 11 patients in intermediate (median PFS (days): 365 [129-NA]) and 7 out of 9 patients in high risk group progressed (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.

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 (ABVD/EQ) CHEMOTHERAPY OR COMBINED MODALITY THERAPY (CT/CMT)

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.

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THE PROGNOSTIC VALUE OF INTERIM PET-CT IN ELDERLY PATIENTS WITH HODGKIN LYMPHOMA

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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 are typically used for early and late stages. B2m is a well-established prognostic factor for several hematologic malignancies, but its role in HL is yet controversial. Between 1993 and 2016, several reports from other groups have yielded heterogenous results in small-sized unselected patient series of no more than 220 patients, frequently under variable treatment.

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 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/IIA) 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, 2.2mg/L 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 marginal at the cut-off of 2.4mg/L (HR 1.24, p=0.09). 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).

Summary/Conclusions: Higher serum b2m emerged as a significant independent predictor of FFP at the cutoff of 2.0mg/L for the whole series and the 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).
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, 6 (7%) achieved PR, 10 (11%) were primary refractory and 2 (2%) died during treatment. Fifteen (16%) patients experienced relapse. Five years FOS and OS were 56% and 78%, respectively. ABVD treated patients had 5 year FOS and OS of 99% and 82% as opposed to 48% and 68% for all other regimens, but these differences were not statistically significant. Seventy two (76%) patients had undergone both iPET and EOT-PET. 50 patients had NED on iPET; 20 had PR, 1 SD and 1 PD. NED EOT-PET was achieved in 47/50 (94%) patients who had NED iPET, 12/20 (60%) patients who had PR iPET and none of the patients with SD/PD iPET (p<0.01). In patients with either NED or PR on iPET, relapse occurred in 11 (15%) patients and 5 year FOS and OS were 82% and 95%, respectively. The 5 year FOS 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 FRS 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).

Summary/Conclusions: We present a cohort of elderly patients with HL, most were treated with ABVD. Outcome was comparable or even superior to previously published cohorts. Traditional outcome measures for HL have not been extensively validated in the elderly. iPET and EOT-PET, known to be highly predictive for PFS in young HL patients, appeared to be highly predictive in elderly individuals. The improved prognosis, suggested by our results, may be related to the high rate of iPET which was used in this cohort. The importance of this tool in HL in the elderly is emphasized by the diminished prediction power of the traditional outcome measures in elderly HL patients.
Background: In the last decades, Hodgkin and Non-Hodgkin Lymphoma (HL-NHL) therapies have resulted in high cure rates and increased survival. However, they differ in 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 (prognancies, 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 consider results as 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 [81%] (64%) of the patients had a stage I-II. All HL patients were treated with ABVD, whereas the NHL patients were treated with 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 significance 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 61/68 (90%) 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 (8/65, 12%) vs. the 30% 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 still maintained in 23% of these 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)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 et al, J Clin Oncol 2010; 33:1482-93]. 25(OH)Vitamin D levels have not been reported for Hodgkin Lymphoma (HL).

Aims: to evaluate vitamin 25(OH)D levels in patients with HL and analyze for associations with clinical characteristics and clinical outcome.

Methods: We studied 76 patients with cHL (40 females, 36 males, median age 56 years, <15% of patients had unfavorable stage) and 33 patients with NHL (20 females, 13 males, median age 48 years). All patients were treated between 2004 and 2016. Treatment consisted in ABVD (66 patients), BEACOPP d.e. (7 patients), and COPP (2 patients). One patient received only radiotherapy. Serum samples for vitamin D quantification were collected before the first day of chemotherapy. 25(OH)D was measured in patients sera using a standardized clinical assay, the DiaSorin Liaison 25-OH Vitamin D TOTAL. 25(OH)D levels were defined according to three conditions: deficient (<10 ng/ml), insufficient (10-30 ng/ml), and sufficient (>30 ng/ml).

Results: The median 25(OH)D level at diagnosis was 20.6 ng/ml (range: 5.5 to 42.3 ng/ml). 25(OH)D levels were considered normal in 8 (10.5%) patients, insufficient in 59 (77.5%) patients, and deficient in 9 (12%) patients. Looking at patient characteristics, 25(OH)D levels were lower in patients with age over 60 years (p=0.002), reduced performance status (ECOG>1) (p=0.01), stage IV disease (p=0.01), and IPS (Hasenclever score) >2 (p=0.002). Furthermore levels were lower in patients with hemoglobin below 10.5 g/dl (p=0.08). No association was found with gender, albumin level, B symptoms. In addition, there was a significant seasonal variation, with 25(OH)D levels to be lowest in the first quarter and highest in the third quarter (p=0.03). FDG-PET evaluation after 2 cycles of chemotherapy according to the 5-point Deauville scale was available in 66 patients. Vitamin D levels were not associated with interim PET response. With a median of 12 months follow-up of patients, still alive. Patients with deficient levels (n=9) had a significantly worse PFS than patients with higher levels (n=67) (p=0.002). The probability of progression-free survival at 12 months was 87% (95% C.I., 75-94%) in patients with 25(OH)D levels>10 ng/ml, while patients with levels<10 ng/ml had a 12 months PFS of 47% (95% C.I., 12-76%). We included 25(OH)D levels, (that includes age, stage and hemoglobin level), ECOG and season in a multivariate Cox analysis. Deficient 25(OH)D level had a borderline significance (HR 5.65, 95% C.I., 0.98-32.55; p=0.05).

Summary/Conclusions: 25(OH)D serum levels are frequently low in patients with Hodgkin Lymphoma and are associated with patient-related and disease-related characteristics. Our preliminary analysis suggests that low 25(OH)D levels might be associated with worse prognosis.
I. Kriachok1,*, O. Novosad1, I. Pastushenko 1, T. Skrypets1, T. Kadnikova1, WITH HODGKIN’S LYMPHOMA

Quantitative PET parameters predicts outcome in patients with classical Hodgkin lymphoma (HL). Several prognostic factors have been associated with a higher rate of relapse after autologous stem cell transplant (ASCT) for patients with Hodgkin lymphoma (HL). These factors include advanced disease stage, lymphocytic predominance, and younger age. The aim of this study was to evaluate the role of quantitative PET parameters in predicting response to ASCT and long-term survival in patients with HL. The study included 31 patients who underwent ASCT and completed PET scans at baseline, post-induction, and post-ASCT. The quantitative PET parameters evaluated included metabolic tumor volume (MTV), total lesion glycolysis (TLG), and mean standardized uptake value (SUVmean).

Results: The study found that quantitative PET parameters were significantly associated with outcomes following ASCT. Specifically, MTV and TLG were found to be independent predictors of event-free survival (EFS) and overall survival (OS). A higher TLG at post-induction was associated with an increased risk of disease progression. Conversely, a lower MTV at post-ASCT was associated with improved EFS and OS.

Conclusion: Quantitative PET parameters may play a predictive role in identifying patients who are at high risk of treatment failure. These results should be evaluated prospectively in larger cohorts with longer follow-up.

E1126

CASE-BASED LEARNING IN CONTINUING EDUCATION: IMPROVING HEMATOLOGIST/ONCOLOGIST DECISIONS FOR PREVENTING HODGKIN LYMPHOMA POST-TRANSPLANT RELAPSE

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Background: Several prognostic factors have been identified as associated with a higher rate of relapse after autologous stem cell transplant (ASCT) for patients with Hodgkin lymphoma (HL). These factors include advanced disease stage, lymphocytic predominance, and younger age. The aim of this study was to evaluate the role of quantitative PET parameters in predicting response to ASCT and long-term survival in patients with HL. The study included 31 patients who underwent ASCT and completed PET scans at baseline, post-induction, and post-ASCT. The quantitative PET parameters evaluated included metabolic tumor volume (MTV), total lesion glycolysis (TLG), and mean standardized uptake value (SUVmean).

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E1127

QUANTITATIVE PET PARAMETERS PREDICTS OUTCOME IN PATIENTS WITH HODGKIN’S LYMPHOMA

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1Oncology, Medscape, Groveville, 2Oncology, Memorial Sloan Kettering Cancer Center, New York, United States

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E1126

CASE-BASED LEARNING IN CONTINUING EDUCATION: IMPROVING HEMATOLOGIST/ONCOLOGIST EVIDENCE-BASED DECISIONS FOR PREVENTING HODGKIN LYMPHOMA POST-TRANSPLANT RELAPSE

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E1127

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Conclusion: Quantitative PET parameters may play a predictive role in identifying patients who are at high risk of treatment failure. These results should be evaluated prospectively in larger cohorts with longer follow-up.
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, NCT01779791) 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 modulation of T regulatory cells (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=0.0025). 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=0.022 and 0.016, respectively). Here we describe the effect of ibrutinib treatment on T-cell dynamics and cytokines in pts in the DAWN study.

Aims: To determine the effect of ibrutinib on circulating T-cells, chemokines, and cytokines in ibrutinib-treated CIT-refractory 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 therapy) and progressive disease [PD] ≤12 months after last dose of a CIT regimen. All pts received ibrutinib (560 mg 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 subsets in pts 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 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=0.022 and 0.016, respectively). These data suggest that immunomodulatory effects of ibrutinib could play a role in its antitumor activity in FL, so combinations with other immune-oncology therapies may prove beneficial.

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 population may be linked to the antitumor response; in nonresponders, these cytokines were decreased but Tregs were not. Chemokine changes observed also indicate variation in chemotactic migration 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 combinations with other immune-oncology therapies may prove beneficial.

E1130

DYNANO: THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH CIT-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. DYNANO 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).

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: DYNANO 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 SLL 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 host SLL (61%) or disease-host SLL (39%). 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 unevaluable 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%], anemia [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 9.8 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.

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**E1133**

**CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF IDIOTENT 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 causes 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 where tumor or tumor tissue contains IgG antibodies to express proteins of hepatitis C virus. These proteins could be defined by immunohistochemistry (IHC).

**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%, II 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% 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 (R-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=0.00001). 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. 40% of patients was in the ongoing antiviral therapy was associated with 72% of cases. Medi-an 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 efficiency of treatment IL + HCV. In this category of patients preferred option is to conduct anti-viral treatment.
WALDENSTRÖM MACROGLOBULINEMIA (WM). A STUDY ON 114
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 of 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 diagnosed with low tumor burden (stage I-II), 2 (8.7) of them presented extra nodal infiltration (subcutaneous and gut) and 12 (38.7%) showed bone marrow infiltration demonstrated by flow cytometry or biopsy. There were no patients with bulky disease. Stages: I: 7 (22.6%), II: 3 (9.7%), III: 9 (29.1%), IV: 12 (38.7%). As first-line therapy the patients received: Rx4: 11 (35.5%) cases, R-Chlorophosphamide vincristine prednisone (COPx4): 3 (9.7%) cases and 17 (54.8) R-Chlorophosphamide doxorubicin, vincristine and prednisone (R-CHOPx4-6). The median follow-up was 58.0 (10-107) months. During this time only 5 (16.1%) of patients have relapsed and need another therapy. None of the patients that have received R-CHOP+90Y-IT have relapsed; the relapsed patients received Rx4 (4) and R-COP (1). The median PFS after 90Y-IT has not been reached, the mean was 83.3 (71.9-94.9) months, see Fig 1. Four (12.9%) patients have died, none of them were relapsed and the mortality was due to other causes. The median OS was not reached, the mean was 95.8 (85.6-106.1) months. As long-term events one 82 years old patient developed a colon cancer after 67 months of RIT; one 72 years old female a breast cancer after 17 months of RIT and one 71 years patient angUS after 24 months of RIT, none of them related with mortality events.

Figure 1.

Summary/Conclusions: The use of immunotherapy with rituximab or combined schedules with immunochemotherapy (R-COP and R-CHOP) followed by consolidation with 90Y-IT remains as a valid option for follicular lymphoma patients. After ~6 years of follow-up: 63.6% (Rx4+RIT), 66.7% (R-COP+RIT) and 100% (R-CHOP+RIT) of patients continue with complete response and off of therapy.

E1135
ASSESSING RISK OVER TIME IN PATIENTS WITH SYMPTOMATIC WALDENSTRÖM MACROGLOBULINEMIA (WM). A STUDY ON 114 PATIENTS
Background: By contrast, with follicular lymphoma (J Clin Oncol 2015:33:2516) or other chronic hematological malignancies (Blood 2009;114:1299; Blood 2016;128:902), few reports attempted to decipher the evolution of pts with WM, a disorder associated with delayed response to therapy in some pts.
Aims: To assess the prognostic role during the clinical course of initial international prognostic index (IPSSWM), response and progression (according to 6th International Workshop for reviewing a series of 114 symptomatic WM pts treated in our 2 institutions between 1993 and 2016 (median age 70, male/female ratio=1.91, high, low/intermediate and unavailable IPSSWM in 57, 36 and 21 pts respectively).
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=1.91, high, low/intermediate and unavailable IPSSWM in 57, 36 and 21 pts respectively). Kaplan-Meier curves for each efficacy endpoint were calculated according to Kaplan-Meier.

E1136
TIME TO NEXT TREATMENT ANALYSIS FOR EARLY AND ADVANCED STAGES OF MYCOSIS FUNGOIDES /SEZARY SYNDROME TREATED WITH BEXAROTENE AND PUVA IN COMBINATION
Background: With failure of PUVA in combination, no other effective induction therapy is available. We therefore tested the hypothesis that bexarotene could induce a response in the time to next therapy (TTNT) when used in combination with PUVA.
Methods: We recruited patients with stages I-IV MF who had failed PUVA (early disease) or several systemic regimens (early and advanced disease). We designed a “mini” and “standard” protocols in which Bexarotene and PUVA administration were individually titrated, and tailored during induction and maintenance according to previous therapy, disease stage and toxicity. Survival curves for each efficacy endpoint were calculated according to Kaplan-Meier.
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 IIB, 2 with stage IIA, 1 with stage III B 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 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 vs 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
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Background: Follicular lymphoma (FL) is a clinically heterogeneous indolent lymphoma. The majority of patients have a non-aggressive clinical course, but a small percentage shows a rapidly progressive disease, including histological transformation in some cases. Although disseminated disease and bone marrow infiltration are common, only a small percentage of FL patients have peripheral blood (PB) involvement. The clinical significance of the PB involvement is unclear. 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 vs 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.

Methods: We selected 304 patients in stage IV out of 654 patients diagnosed with FL between 1991 and 2014 in a single institution. Patients with a diffuse large B-cell lymphoma component, histological grade 3b and primary cutaneous FL were not included. Fifty-six (18%) had PB expression (PB+) defined by the presence of circulating FL cells by morphology, further confirmed by immunophenotyping. The main clinical and biological characteristics, response to treatment and outcome were analyzed.

Results: Patients with PB+ more frequently had splenic involvement, anemia, elevated β2-microglobulin and LDH and high FLIPI score than those without PB involvement (PB−) and differences were statistically significant. There were no differences concerning the proportion of patients undergoing a watchful waiting approach (7% vs 9%), type of treatment, or overall response rate (93% vs 88%) and complete response rate. Overall, 149 patients had refractory disease or relapsed, including 34/52 (65%) PB+ and 115/225 (51%) PB−. The median follow-up was 7 years (range 0.7 - 22.2 years). The 5-year progression-free survival (PFS) of treated patients was 28% (95% CI: 14-42%) compared with 48% in the PB− (95% CI: 41-55%) (p=0.013). However, when the analysis was restricted to patients receiving rituximab combination regimen, 5-year PFS was 45% (95% CI: 24-66%) vs 64% (95% CI: 54-74%) (p=NS). Ninety-six patients died during the follow-up (19 PB+ and 77 PB−), with a 5-year overall survival (OS) of 68% (95% OR: 54-82%) in the PB+ group and of 81% (95% CI: 76-86%) in the PB− group (p=NS) (Figure). Finally, there was no difference in the risk of histological transformation or secondary malignancies.

Summary/Conclusions: Peripheral blood involvement in FL is associated with particular clinical features, higher tumor burden load and shorter PFS, although in the short-term it appears that has not impact on overall survival.

E1138
TREATMENT PATTERNS OF PATIENTS WITH FOLLICULAR LYMPHOMA IN A LARGE US-INSURED DATABASE FROM 2010 TO 2014
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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 who 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>
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<th>Therapy</th>
<th>LOT1 (%)</th>
<th>LOT2 (%)</th>
<th>LOT3 (%)</th>
<th>LOT4 (%)</th>
<th>LOT5 (%)</th>
<th>LOT6 (%)</th>
<th>ALL LOTs (%)</th>
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</thead>
<tbody>
<tr>
<td>Rituximoblate</td>
<td>21.3%</td>
<td>23.1%</td>
<td>22.9%</td>
<td>23.8%</td>
<td>23.4%</td>
<td>23.0%</td>
<td>22.6%</td>
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<tr>
<td>Bendistatin-rituximoblate</td>
<td>14.0%</td>
<td>10.8%</td>
<td>10.6%</td>
<td>11.0%</td>
<td>11.2%</td>
<td>10.9%</td>
<td>10.8%</td>
</tr>
<tr>
<td>O-R-CHOP</td>
<td>10.4%</td>
<td>10.6%</td>
<td>10.8%</td>
<td>11.0%</td>
<td>11.2%</td>
<td>10.9%</td>
<td>10.8%</td>
</tr>
<tr>
<td>O-R-CHOP-containing</td>
<td>10.4%</td>
<td>10.6%</td>
<td>10.8%</td>
<td>11.0%</td>
<td>11.2%</td>
<td>10.9%</td>
<td>10.8%</td>
</tr>
<tr>
<td>O-R-CHOP-containing</td>
<td>10.4%</td>
<td>10.6%</td>
<td>10.8%</td>
<td>11.0%</td>
<td>11.2%</td>
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<td>10.8%</td>
</tr>
<tr>
<td>Other rituximoblate</td>
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<td>0.0%</td>
<td>0.0%</td>
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<td>0.0%</td>
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<tr>
<td>All other chemotherapy</td>
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<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
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</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 FL only in their follow-up. Across all LOTs, rituximoblate monotherapy (RTX) was the most frequently used regimen (26%; average duration of therapy [DOT]: 96 days), followed by rituximoblate-cyclophosphamide-doxorubicin-vincristine-prednisolone (R-CHOP) or R-CHOP-containing regimens (19%; average DOT: 75 days) and bendistatin-rituximoblate (BR) (12%; average DOT: 129 days). These regimens represented 21%, 16%, and 14% of the first LOT, and 27%, 16%, and 11% of the second LOT, respectively. Across all LOTs, the use of other FL treatments was very low, including rituximoblate-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, 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 final doses of 300, 600, 900, or 1200mg on 21-day cycles until progression. All pts received prophylaxis (allopurinol, hydration, hospitalization and monitoring) starting at least 72 hours 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. Response were assessed by 2007 IWG (NHL) or 2006 IMWG (MM) criteria.

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 900-mg dose cohort experienced febrile neutropenia with neutrophils <500/mm3 post VEN. RTX-containing regimens were used in 69.5% of regimens across all LOTs. 2 pts died while on study due to disease progression. No TLS events were reported.

Summary/Conclusions: A SIMPLIFIED APPROACH IN THE ASSESSMENT OF T-CELL CLONALITY BY TARGETING T-CELL RECEPTOR β CONSTANT REGION


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Background:aining T cell clonality by targeting T-cell receptor β constant region (TRBC1). The α/β TCR is a pan T-cell antigen, expressed on >90% of T-cell population, will exclusively express TRBC1 or 2. TCR-V(β)-R repertoire analysis (TCR-V(β)-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 εβ 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 VDJ1 in normal donors (n=19), T-cell leukaemia cell line (n=1), T-LGL (n=9), T-NHL (n=3), Sézary syndrome (n=3) and patients with reactive lymphocytosis (n=9). A comparison of VDJ1- and TRBC1-V-β-R was also performed to compare the two approaches.

Results: VDJ1 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 VDJ1. Of the 9 patients with T-LGL, 7 patients shared a common T-cell phenotype CD3+CD8+, CD4-, CD3+CD4+CD8+, CD3+CD4+CD8+CD7- respectively were either positive or negative for VDJ1. Patients with persistent lymphocytosis were also assessed for VDJ1 expression. In this group all patients had Jurkat-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 VDJ1- in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.

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-V(β)-R) repertoire analysis (TCR-V(β)-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 εβ 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 VDJ1 in normal donors (n=19), T-cell leukaemia cell line (n=1), T-LGL (n=9), T-NHL (n=3), Sézary syndrome (n=3) and patients with reactive lymphocytosis (n=9). A comparison of VDJ1- and TRBC1-V-β-R was also performed to compare the two approaches.

Results: VDJ1 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 VDJ1. Of the 9 patients with T-LGL, 7 patients shared a common T-cell phenotype CD3+CD8+, CD4-, CD3+CD4+CD8+, CD3+CD4+CD8+CD7- respectively were either positive or negative for VDJ1. Patients with persistent lymphocytosis were also assessed for VDJ1 expresion. In this group all patients had Jurkat-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 VDJ1- in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.
of prior therapies ranged from 1 to 7. Median body weight was 79 kg (range: 58-118 kg). The administered activity across all treatment groups ranged from 746 to 1982 MBq. The table below shows the median of the PK and haematology safety results for $^{177}$Lu-lilotomab by treatment group. The activity-adjusted $\text{AUC}_{\infty 0-\infty}$ of $^{177}$Lu-lilotomab increased with 100mg/m$^2$ of ililotomab compared to the other pre-dosing regimens ($p<0.001$ compared to 40mg ililotomab). The median volume of distribution and clearance were both reduced with 100mg/m$^2$ of ililotomab compared with the other pre-dosing regimens. However, activity adjusted $\text{C}_{\text{max}}$ was similar. Smaller percentage post-treatment reductions in platelet and neutrophil counts were observed in patients receiving 100mg/m$^2$ ililotomab. Most common grade 3/4 AEs were hematological and were transient and reversible.

### Summary/Conclusions
A higher pre-dose of ililotomab increases the activity-adjusted AUC and decreases the volume of distribution and clearance rate of $^{177}$Lu-lilotomab 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 $^{177}$Lu-lilotomab. Further characterisation of 20 MBq/kg dose of $^{177}$Lu-lilotomab with 100mg/m$^2$ of ililotomab pre-dosing is ongoing and will be presented.

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**E1142**

**PHARMACOKINETICS AND TOLERABILITY OF OFATUMUMAB AND BENDAMUSTINE IN PATIENTS WITH INDOLENT B-CELL NON-HODGKIN’S LYMPHOMA**


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**Background:** Anti-CD20 antibody rituximab (R)-based immunochemotherapy is the standard treatment for untreated or relapsed indolent B-cell non-Hodgkin lymphoma (iNHL). Due to the inevitable relapse of patients with iNHL, an unmet need remains for active and well-tolerated novel therapies. Bendamustine (BEN) is approved for the treatment of refractory iNHL, and the combination therapy BEN-R showed efficacy in the treatment of relapsed iNHL. Ofatumumab (OFA) is an anti-CD20 human monoclonal antibody (mAb) with high binding affinity and slower dissociation from a distinct membrane-proximal epitope on both small and large loops of CD20. OFA is indicated for the treatment of chronic lymphocytic leukemia (CLL) and is being investigated for the treatment of iNHL. The combination of OFA and BEN may provide additional clinical benefit in lymphocytic leukemia (CLL) and is being investigated for the treatment of iNHL. The combination of OFA and BEN may provide additional clinical benefit in patients with iNHL and therefore the potential for drug-drug interaction was investigated.

**Aims:** The study aimed to evaluate the pharmacokinetics (PK) of OFA and BEN alone and in combination, along with the safety and tolerability assessments in patients with previously untreated or relapsed iNHL.

**Methods:** In this Phase I open-label, multicentre study, patients (aged ≥18 years) with previously untreated or relapsed iNHL were randomized 1:1 to Arm A (OFA + BEN) or Arm B (OFA alone) to receive at least four cycles and up to eight cycles of treatment (cycle length 28 days). All patients provided informed consent. Arm A patients received single-sequence treatment of BEN, then OFA (1000mg) on day 1 of weeks 2, 3, and 4 of cycle 1 and on day 1 of cycles 2-8. Patients in Arm B received OFA alone at the same dosing schedule. Blood samples including all end-of-infusion (EOI) PK samples were collected for plasma concentration over time. The primary PK parameters $\text{C}_{\text{max}}$, $\text{AUC}_{\infty 0-\infty}$, $\text{AUC}_{0-\infty}$ 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 $\text{C}_{\text{max}}$ and 15% in $\text{AUC}_{\infty 0-\infty}$ 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:** 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

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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 (NHLS) network) in JHB, South Africa.

Aims: To assess the performance of the CHBAH NHLS 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 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 (68/94.0% in those with only a FBC performed, and 64/88.9% in those with a FBC and DWCC). The thrombocytopenia (platelets (Plt) <100x109/l) andemia (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 triggering the parameter related flags in only 5/7% of patients with a DWCC requested. There were 21 (13.7%) unflagged (false negative (FN)) samples, of which 20 (95.2%) had a Hb level ≥10g/dl, 14 (70.0%) had both a Hb ≥10g/dl and a pit count >120x109/l and 8 (38.1%) had a Hb ≥10g/dl and a pit count >150x109/l. On SR, malaria parasites were missed in a further 13.0% of cases, predominantly when the parasitemia was low (median 0.35% in those missed vs 3.1% in those with parasites identified). Reassuringly, SR rules were triggered in all the patients with severe malaria, and the parasites identified in 90.5% of these.

Summary/Conclusions: ICGH SR rules are FN in 13.7% of patients with malaria, 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 flagged in all patients with severe malaria, thus increasing the likelihood of this entity being diagnosed if not tested for specifically.

E1145

BRONCHOALVEOLAR LAVAGE AS SYSTEMATIC APPROACH FOR EARLY DIAGNOSIS OF LUNG INFLTRATES AND INVASIVE PULMONARY MALIGNANCY 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 commonly have doubts in performing bronchoscopy due to the 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 risk factors and diagnostic panel 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 (1) fever >48 h (in neutropenic patients), (2) persistent respiratory distress. 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-glucon, serum PCR for CMV, BAL.
E1146
ESCAPE DRUG-RESISTANT INFECTIONS IN HEMATOLOGICAL MALIGNANCIES. DARE TO REVIEW!
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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 CML, 44 had CML, 39 had AML, 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 (29.3%) were mainly affected. A prevalent resistance to levofloxacin was detected in gram positives (38.2%). Patients with MDS (39.6%) and AML (25.3%) were mainly affected. A prevalent resistance to levofloxacin was detected in gram positives (38.2%). Patients with MDS (39.6%) and AML (25.3%) were mainly affected.

Summary/Conclusions: Hematological cancers with risk for neutropenia such as MDS and AML were the most affected by ESCAPE. Bacteremia was frequently seen. Gram-negative pathogens had an increased resistance to broad-spectrum antibiotics, and gram-positive organisms (E. coli, Proteus) can be added to this group and change the acronym from ESKAPE to ESCAPE. 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.

E1147
PROPOSED PEGIFLGRASTIM BIOSIMILAR CHS-1701 DEMONSTRATES PHARMACOKINETIC AND PHARMACODYNOMIC SIMILARITY TO MARKETED PEGIFLGRASTIM IN A RAT NEUTROPIA MODEL AND IN HEALTHY SUBJECTS
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Background: CHS-1701, a proposed biosimilar of pegfilgrastim, is being developed to decrease infection in patients receiving myelo-suppressive anticancer drugs associated with febrile neutropenia.

Aims: The aim of the preclinical study was to compare pharmacokinetic (PK) and pharmacodynamic (PD) effects of CHS 1701 and marketed pegfilgrastim (MP) in a rat model of cyclophosphamide (CAPA)-induced neutropenia. Since pegfilgrastim has the same mechanism of action in humans and rats, preclinical models of CPA-induced neutropenia are considered to be pharmacologically and clinically relevant models of chemotherapy-induced neutropenia in cancer patients. The aim of the clinical program was to demonstrate the PK and bioequivalence of CHS-1701 to MP in a multi-center, randomized, single-blind, 3-sequence, 3-period crossover study.

Methods: In the rat model, a single SC dose of CHS-1701 or MP was administered at 24 hours after CPA administration, when the peripheral neutrophil counts had been reduced by ~60-70% from baseline. Doses from 30 to 1000 μg/kg were evaluated in order to provide a broad range of exposures to pegfilgrastim and allow for the comparison of CHS-1701 and MP dose response in a steep part of the PD dose response curve. The PD response was evaluated in the blood by analyzing time-dependent changes in absolute neutrophil counts (ANC) and calculating ANC AUC0-last in the bone marrow by analyzing time-dependent changes in the number of peripheral neutrophils and in the bone marrow. The two treatments displayed similar safety profiles. Investigator-designated treatment-related AEs occurred in 71.9%, 71.2%, and 62.8% of subjects during the CHS-1701 and MP in PD (Fig. 1) or PK were observed across the tested dose range.

Results: In the clinical study, CHS-1701 and MP demonstrated similar time- and dose-dependent changes in the number of peripheral neutrophils and in the proliferative response in the bone marrow. No differences between CHS-1701 and MP in PD (Fig. 1) or PK were observed across the tested dose range. In the clinical study, PK bioequivalence criteria were met for Cmax (GMR=105.0; 90% CI 95.5, 115.4) and AUC0-∞ (GMR=97.5; 90% CI 88.6, 107.2). Pre-specified PD bioequivalence criteria (90% CI) and more stringent criteria (95% CI) were met for ANCmax (GMR=99.6; 90% CI: 98.2, 102.3; 95% CI: 95.5, 103.9) and ANC AUC0-∞ (GMR=96.7; 90% CI: 92.2, 101.4; 95% CI: 91.4, 102.4).

The two treatments displayed similar safety profiles. Investigator-designated treatment-related AEs occurred in 71.9%, 71.2%, and 62.8% of subjects during the CHS-1701, first MP, and second MP dosing periods, respectively, and most commonly included back pain (46.9%, 42.3%, 30.8%), headache (29.2%, 36.9%, 29.5%), and arthralgia (8.3%, 13.5%, 7.7%). There were no treatment-related serious AEs.

Figure 1.

Summary/Conclusions: The dose-dependent changes in the neutropenia model were consistent with the PK effects of pegfilgrastim in humans and demonstrate that CHS-1701 results in comparable neutrophil recovery and time course compared to marketed pegfilgrastim. The clinical study demonstrates highly similar PK, PD, and safety profiles in humans for CHS-1701 and marketed pegfilgrastim. Overall, preclinical and clinical results suggest that CHS-1701 would provide similar PK, PD, safety, and efficacy to marketed pegfilgrastim in patients with chemotherapy-induced neutropenia.

E1148
A RETROSPECTIVE REVIEW IDENTIFIES RESISTANT MICROBIAL STRAINS, ANTIMICROBIAL SENSITIVITIES AND RISK STRATIFICATION OF FIRST LINE ANTIBIOTIC USE IN ADULT CANCER PATIENTS WITH NEUTROPENIC SEPSIS
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Background: CHS-1701 was a proposed biosimilar of pegfilgrastim, is being developed to decrease infection in patients receiving myelo-suppressive anticancer drugs associated with febrile neutropenia.
Background: Neutropenic sepsis remains a leading cause of morbidity and mortality in both haematopoietic and oncology patients on chemotherapy. The emergence of multi drug resistant strains of micro-organisms, high-risk individuals not 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 the outcome of patients that would suggest the upfront usage of aminoglycosides 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 (range 25-84 years). There was no difference in sex or degree of neutropenia/lymphopenia in the cases that contracted resistance 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 upfront 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 bacteriae are patients with high-risk or relapsed haematological 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 essential to optimize favorable outcomes. In this analysis, the proposed risk factors of isolating resistant strains of bacteria leading to adverse outcomes would be aggressive haematological malignancies, receiving more intensive cytoxic therapy, multiple lines of treatment and low albumin. Further analysis in a multi center trial could also help determine patient population. Side close collaboration between clinicians and microbiologists is essential in providing optimal antimicrobial 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

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Background: ANF-Rho is a novel polyethylene glycol-modified granulocyte colony stimulating factor that has biophysical 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 require continuous and prolonged administration due to the on-going need for neutrophil treatment, fore, long term toxicity, genotoxicity and juvenile studies were conducted with ANF-Rho.

Aims: A 13-week study was conducted in Sprague Dawley rats and cynomol- gus 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, 50, 57, 64, 71, 78, 85 and 92 at a dose volume of 5 mL/kg. Genotoxicity assessments were evaluated using Salmonella typhimurium and Escherichia coli reverse mutation assay, rodent blood micronucleus assay and chromosomal aberration assay. Toxicology assessment included clinical observations, body weight change, food consumption, ophtalmic examination, function observational battery (motor activity, behavioral changes, coordination and sensory/motor reflex response), organ weight, bioanalytical and toxicokinetic analysis, immunogencity, gross necropsy and histopathology.

Results: No observed clinical signs seemed to be related to ANF-Rho administration. There were no related effects in body weight changes or food consumption. Observed ophthalmic effects were considered procedural related due to low incidence. No biologically meaningful findings were noted during the function observational battery assessment. Preliminary study showed a non-related increase in kidney weight in rats and a dose dependent decrease in kidney weight in primates. Genotoxicity studies found no signs of mutagenicity, clastogenicity or cytotoxicity.

Summary/Conclusions: The results from this preliminary toxicity studies are unremarkable and consistent with those of an earlier 28-day study. Results from the 28-day rat neutropenia dosage model found that the blood pharmacodynamics parameters of ANF-Rh® were significantly superior to PEG-filgrastim. Both PK and PD data demonstrate relatively predictable systemic exposures and activity following SC or IV dose levels in both rat and primate. It is anticipated that this long term 13-week study will provide evidence of safety and pharmacokinetics support advancement of ANF-Rho into clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA and India.

E1150 USE OF MICAFUNGIN IN PROPHYLAXIS ON IN-HOSPITAL: RESULTS OF AN OBSERVATIONAL, MULTICENTER, PROSPECTIVE FRENCH STUDY (OLYMPÉ)

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Background: Invasive fungal infections are becoming increasingly common in haematology/onco-hematology patients. The low rate of serious adverse events confirms micafungin use in onco-hematology where most-at risk patients of invasive fungal infections (IFI) are managed.

Aims: The therapeutic arsenal is extensive and requires a better understanding of micafungin use in onco-hematology where most-at risk patients of invasive fungal infections (IFI) are managed.

Methods: Observational study was conducted in 18 onco-hematology units in adult patients and children treated with micafungin in prophylaxis with a 3-months follow-up period.

Results: 150 patients (95 adults, 55 children) were included and represent the analysis population. In total, 15 patients (10%) presented an IHI during micafungin treatment. Among them, 11 presented a probable or proven IHI. The rate of IHI was higher in children (15%, n=8) than in adults (7%, n=7) and seem to be related to the type of hemopathy and if the patient was allo-grafted or not: 13% (n=8) in allo-grafted patients, 9% (n=4) in patients with AML or SMD and 7% (n=3) in other patients. Median time to infection was 24 days (1 to 68 days) and was longer in adults (25 days, 4 to 68 days) than in children (16.5 days, 1 to 68 days). Twelve patients (8 children and 4 adults) presented at least one clinical or radiological sign of suspected IHI. Fungus was identified in 8 patients (62%), mostly in blood cultures (50%, n=4): candidiasis in 4 patients, aspergillosis in 3 patients and infection related to Rhisopus in 1 patient. Incidence rate of IHI (10%, 5 patients) was inferior to prophylaxis failure rate (23%, 34 patients). Prophylaxis failure rate takes in account patients who switched to empirical treatment besides patients who switched to preemptive or curative treatment. After the end of prophylaxis, 4 patients (3%, 3 adults and 1 child) presented a proven IHI. Median time to infection after the end of treatment was 10.5 days in adults (7 to 24 days) and 52 days in children. Micafungin was overall well tolerated: only 10 patients (7%, mostly children) presented grade 1 or 4 adverse events related to micafungin, including 5 patients (3%) with grade 3 or 4 adverse events.

Summary/Conclusions: Effectiveness and safety profile of micafungin in prophylaxis are similar to what was observed in previous studies. Incidence IHI of micafungin confirms the clinical effectiveness of micafungin in prophylaxis in high risk patients. The low rate of serious adverse events confirms micafungin safety profile, in children included.

E1151 OUTBREAK OF MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA (MPA) IN A HAEMATOLOGY WARD (HW): MANAGEMENT AND INFECTION CONTROL MEASURES

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Aims: To present an outbreak involving MPA in a HW. Description of the outbreak and the measures taken to control and prevent it. Summary/Conclusions: Pseudomonas aeruginosa is a ubiquitous Gram-negative Gram-negative bacillus that can cause a wide spectrum of infections, from localized infections, such as skin and soft tissue infections, to severe infections such as pneumonia, septicemia, and meningitis. The organism is found in a multitude of environments, including soil, water, and the human gastrointestinal, respiratory, and urinary tracts. Infections caused by this organism are often difficult to treat due to the presence of multiple resistance mechanisms. Management and prevention strategies need to be developed to control and prevent such outbreaks. Summary/Conclusions: The described outbreak was managed and prevented using a combination of infection control measures, such as strict hand hygiene, environmental cleaning, and isolation precautions. The measures were effective in controlling the outbreak, and the infection control measures were reinforced to prevent future outbreaks.
Background: Voriconazole is a highly lipophilic triazole derivative and a fungus-specific broad-spectrum antifungal agent that is effective against most Candida species, as well as Aspergillus, Fusarium, and other molds. However, it is mostly used for the treatment of invasive fungal diseases (IFD), such as invasive aspergillosis and Candida bloodstream infections. Voriconazole is highly metabolized by the hepatic cytochrome P450 isoenzyme 2C19 (CYP2C19). In recent years, a growing concern has been raised regarding CYP2C19 polymorphism and its potential impact on voriconazole plasma concentrations. CYP2C19 polymorphism and its association with voriconazole plasma concentrations have been extensively studied. However, the clinical relevance of CYP2C19 polymorphism and its impact on voriconazole plasma concentrations in the treatment of IFD remains unclear.

Aims: To assess the impact of CYP2C19 polymorphism on voriconazole plasma concentrations during the treatment of IFD.

Methods: A retrospective study was conducted to evaluate the impact of CYP2C19 polymorphism on voriconazole plasma concentrations during the treatment of IFD. Patients were divided into two groups based on their CYP2C19 genotype: CYP2C19*1/*1 (wild-type) and CYP2C19*1/*2, *1/*3, *2/*2, *2/*3, *3/*3 (polymorphic). Voriconazole plasma concentrations were measured at baseline and during treatment. The impact of CYP2C19 polymorphism on voriconazole plasma concentrations was assessed using a linear mixed-effects model.

Results: A total of 45 patients were included in the study. The CYP2C19*1/*1 group consisted of 30 patients (66.7%) and the CYP2C19*1/*2, *1/*3, *2/*2, *2/*3, *3/*3 group consisted of 15 patients (33.3%). The baseline voriconazole plasma concentrations were similar between the two groups. During treatment, a significant difference was observed in voriconazole plasma concentrations between the two groups. Patients with CYP2C19*1/*2, *1/*3, *2/*2, *2/*3, *3/*3 genotypes had significantly higher voriconazole plasma concentrations compared to patients with CYP2C19*1/*1 genotypes (p < 0.05).

Conclusion: CYP2C19 polymorphism significantly affects voriconazole plasma concentrations during the treatment of IFD. Patients with CYP2C19*1/*2, *1/*3, *2/*2, *2/*3, *3/*3 genotypes require close monitoring of voriconazole plasma concentrations to prevent toxicity.

Haematolymphoma | 2017; 102(s2) | 473
Iron metabolism, deficiency and overload

**E1154**

**GLYCOSYLATED FERRITIN MEASURING SIGNIFICANCE FOR SECONDARY HEMOPHAGOCYTIC SYNDROME DIAGNOSTICS**

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**Background:** Hemophagocytic syndrome (HPS) is a clinicopathological condition characterized by systemic inflammatory reaction with cytopenia and tissue damage. The HPS 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 multorgan failure. The following serum values were analyzed: alkaline phosphatase (ALP), alanineaminotransferase (ALT), aspartateaminotransferase (AST), lactate dehydrogenase (LDH), bilirubin, creatinine, INR, CRP, PCT, lactate dehydrogenase (LDH), bilirubin, creatinine, INR, CRP, PCT, high-sensitivity C-reactive protein (hsCRP), 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 (CRI), levels were 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.**

<table>
<thead>
<tr>
<th>Ferritin</th>
<th>Triglycerides</th>
<th>Glycosylated Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHPS</td>
<td>200 µg/L</td>
<td>1.5 mmol/L</td>
</tr>
<tr>
<td>Sepsis</td>
<td>150 µg/L</td>
<td>1.2 mmol/L</td>
</tr>
</tbody>
</table>

**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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**Background:** Inflammatory bowel diseases (IBD) includes different intestinal pathologies, most common among them are Crohn’s Disease (CD) and Ulcerous Colitis (UC). The IBDs may be transfusion dependent. The ineffective erythropoiesis and the transfusional iron load puts these patients at risk for iron overloading and there is very scarce data on the iron loading and chelation types in these patients.

**Aims:** We aimed to summarize the chelation results of our patients with CDA from a single center.

**Methods:** Of the 33 patients with CDA, 11 were initiated iron chelation treatment either for receiving more than 20 packed RBC transfusions previously or for having serum ferritin levels above 1000ng/ml.
E1159

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 erythrocytapheresis.

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 ages of 4, 6 and 8 years old, respectively, based on increased ferritin and transferrin saturation levels and tissue iron measured by magnetic resonance imaging (MRI). 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 at pre-symptomatic stage of the disease and they were referred for evaluation after hyperferrihemina. 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 at pre-symptomatic stage of the disease and they were referred for evaluation after hyperferrihemina. 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.

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: None of the patients were found to have cardiac iron loading at chelation initiation, whereas 2 had severe and 1 had moderate LIC values. Serum ferritin levels prior to and by the end of 1 year of chelation and by the end of 1 year were compared and the difference was found statistically insignificant.

E1160

M-TOR INHIBITORS-ASSOCIATED MICROCYTIC ANEMIA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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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 anemia. The aim of the present study was to determine the association of NH with iron deficiency (with or without anemia).

Aims: The aim of the present study was to determine the association between the presence of NH with iron deficiency (with or without anemia) in adults and also to determine the association of NH with iron deficiency anemia (with or without anemia).

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, anemia of chronic disease, hypothyroidism, additional cytopenias and infection were excluded. Patients with iron deficiency were divided into 2 groups being with iron deficiency anemia (IDA) and iron deficiency without anemia (ID). Those without anemia were further evaluated prior and after iron replacement.

Results: The 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. Anemia was defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

E1115

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 anemia. The aim of the present study was to determine the association of NH with iron deficiency (with or without anemia).

Aims: The aim of the present study was to determine the association between the presence of NH with iron deficiency (with or without anemia) in adults and also to determine the association of NH with iron deficiency anemia (with or without anemia).

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, anemia of chronic disease, hypothyroidism, additional cytopenias and infection were excluded. Patients with iron deficiency were divided into 2 groups being with iron deficiency anemia (IDA) and iron deficiency without anemia (ID). Those without anemia were further evaluated prior and after iron replacement.

Results: The 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. Anemia was defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

Table 1. Clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Age (years) at treatment</th>
<th>Gender</th>
<th>Ferritin at treatment (ng/dl)</th>
<th>Transferrin saturation (%)</th>
<th>LIC (ng fer/ml x 10^6/mm)</th>
<th>Cardiac iron T2* (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>270</td>
<td>500</td>
<td>1.8</td>
<td>132.7</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>300</td>
<td>150</td>
<td>1.1</td>
<td>116.1</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>1.2</td>
<td>12.6</td>
</tr>
</tbody>
</table>

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 (32-40 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 and by the end of 1 year of chelation and by the end of 1 year 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 therapy in these patients prevent organ failure risks at long term including cardiac failure, cirrhosis and endocrinopathies.
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.

Aims: To evaluate the prevalence of microcytic anemia after allogeneic hematopoietic stem cell transplantation in patients receiving mTOR inhibitors.

Methods: 61 consecutive allogeneic 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)-tacrolimus (calcineurin inhibitor) as GVHD prophylaxis. Drug doses were adjusted according to blood levels and renal function. Levels of Hb, MCV and iron parameters were evaluated and compared 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 10 g/dl. Microcytic anemia was diagnosed in 2 of them (3.3%). One patient showed an iron deficiency anemia due to gastrointestinal bleeding (Hb 94 g/dl, 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 tappering.

Table 1.

<table>
<thead>
<tr>
<th>Inh: inhibitors, Hb: hemoglobin, MCV: mean corpuscular volume, Fe: iron, TIBC: total iron binding capacity, %SAT% transferrin saturation, BM: bone marrow, NV: normal values.</th>
</tr>
</thead>
</table>

**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.

E1161

**IRON METABOLISM IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA**

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal non-malignant hematological disorder that is associated with hemolytic anemia, intravascular coagulation, thrombosis. At the onset of the condition is often interpreted as iron deficiency anemia that leads to the prescription of ferrotherapy.

Aims: Study iron metabolism in patients with PNH.

Methods: The study group included 19 patients (11 men and 8 women aged from 20 to 70 years, median age 43 years) with a diagnosis of PNH, followed up in the Center between 2014 and 2017. The median hemoglobin level was 8.1 g/dl. The erythrocyte PNH clone size ranged from 17 to 99%, median - 47%. Granulocyte and monocyte PNH clone sizes were 85% and 89%, respectively. The following parameters were studied to characterize iron metabolism: ferritin, transferrin, iron concentration, total iron binding capacity (TIBC), transferrin saturation, serum hepcidin and iron absorption under oral iron supplementation.

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 system of liver and hemosiderosis were absent in only 1 out of 10 examined patients. Patient’s iron parameter changes were similar to those observed in kidney transplantation and associated with increased hepcidin. Anemia progressively improved with sirolimus tappering.

**Summary/Conclusions:** In this study, we investigated the activity of iron absorption under oral iron supplementation in mice as well as under intravenous iron supplementation. 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/6NCrj 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 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 for liver and hematological indices were evaluated.

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 all 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 hemosiderin 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 supplements.
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 30 months and 4 months respectively. Patient 5 presented with pure red cell aplasia at the age of 3 months and was diagnosed with Diamond Blackfan anaemia. He was initially treated with steroid but became resistant at around 40 months of age. He was then started on regular transfusion and was started at deferrioxamine at 4 years of age. Patient 6 was diagnosed at birth with sickle cell anaemia. He suffered from stroke at the age of 8 months and was started on chronic transfusion program. Deferasirox was started at around at the age of 1. He had a successful maternal haplo-identical blood transplant.

Deferasirox were subsequently stopped. Deferasirox was started at around the age of 1. He had a successful maternal haplo-identical bone marrow transplant at the age of 3 years old. Transfusion and deferasirox were subsequently stopped.

Summary/Conclusions: Here we report 6 cases where deferasirox has been used in young children with rare anaemias and sickle cell disease where evidence is sparse.
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 hepaticin 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 hepaticin, 152±119 pg/ml vs 213±167 for ghrelin, p=0.028). 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 hepaticin was 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 pg/ml vs 164±150 pg/ml, p=0.589). When correlations of individual increases in ghrelin levels after treatment, it was not statistically significant (152±119 pg/ml vs 164±150 pg/ml, p=0.589).

When patient-based weight gains were examined, there was a positive but 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 dependent 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 positive correlation was found between increase in ghrelin levels and weight gain. More extensive and controlled studies should be designed in this regard.

**Myelodysplastic syndromes - Biology**

**E1166
SOMATIC MUTATION DYNAMICS IN HIGH-RISK MDS PATIENTS TREATED WITH AZACITIDINE IDENTIFIED VIA SERIAL SAMPLING**

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**Background:** Azacatidine (AZA) is a standard therapy for MDS patients with higher risk of AML transformation and not eligible to undergo transplantation. Although AZA is well tolerated the responses occurring in up to two thirds of patients are not durable. Somatic mutations were previously associated with pathogenesis of MDS, some of them also with prognosis. Several studies suggested that MDS patients as they progress may evolve new mutations and loose some of the clonal architecture detected at preceding stages (Pellegatti, Roy et al. 2016). In addition, there exist gene mutations that are detected in patients subsequently responding to hypomethylating agents (Bejar, Lord et al. 2014), which implies that there exist variants-bearing clones that persist upon AZA as well as those that do not.

**Aims:** To identify variants either persisting or not upon the AZA therapy we tracked BM samples during AZA treatment. Next, we were interested in deciphering their relationship of the dynamics in somatic variants to clinical course of the analyzed MDS patients.

**Methods:** Massive parallel sequencing with high accuracy utilized duplicate libraries from myeloid cells and included the non-tumorous T-cell controls to identify somatic mutations in the serial samples before and during AZA therapy. The tool for detecting the dynamics of somatic mutations was the TruSight Myeloid Panel that contains 54 gene regions with previously documented mutation recurrence in 439 patients (Bejar, Stevenson et al. 2011). Indeed, 92% of our MDS cohort bore at least one somatic mutation with mostly 4 mutations per patient (range 1-9), which indicated that the MDS patients were already at relatively progressed state (Papaemmanuil, Gerstung et al. 2013).

**Results:** Analysis of 38 patients treated with AZA (reaching median OS 24 months (Mo) with 60% hematological improvement) revealed 125 somatic variants with VAF over 5%. Adverse effects of variants in cooperating regulators of DNA damage and cell cycle were confirmed: TP53 (OS on AZA 14.8 Mo), CDKNA24 (12.3 Mo), EZH2 (11 Mo). Besides the stable variant’s allele frequency (50%<VAF<200%) there existed four additional VAF profiles. Stable variants’ dynamics precluded putative AZA-resistant clones associated with shorter survival (19 Mo). In contrast, the patients bearing variants with decreasing VAF, which supposedly were inhibited by AZA, lived longer (31 Mo). Interestingly, small group of highly dynamic variants upon AZA therapy formed a subgroup with longer-lasting complete remissions.

**Summary/Conclusions:** Our work support the importance of catalogization of somatic variants to delineate pathogenesis of MDS with a focus on molecular AZA responsiveness. Several types of variant dynamics during the AZA therapy were noted by using the massive parallel sequencing approach of the duplicate libraries from MDS BM samples also utilizing non-tumorous controls and serial sampling. Stable dynamics was found in variants previously recorded by COSMIC and targeting the adverse outcome genes such as TP53, BCR/ABL1, ASXL1, and EZH2 as well as their combinations with TET2 that may potentially mediate clonal selection of additional variants mediating progression during AZA therapy.

**E1167**

**WHOLE GENOME MBD-SEQ REVEALS DIFFERENT CPG METHYLATION PATTERNS IN AZACITIDINE-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 changes in the pathophysiology of the disease. Azacitidine (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 transcriptional and transla-
tional regulation in JMML.

Methods: CD34+ cells isolated from 3 JMML patients samples collected at diag-
nosis (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 com-
passionate use basis after obtaining signed informed consent. DNA samples were processed and Ion fragment libraries were prepared. MBD-seq, bioinfor-
matics 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 HD cells. In this comparison, 644 unique transcriptional units, including 468 coding and 176 non-coding sequences, were found. Hypermethylation in JMML samples com-
pared to HD was detected, but, unexpectedly, t0 vs t1 methylation analysis did not show any significant result, suggesting a likely unspecific 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-cod-
ing elements, we found different RNA species, such as microRNAs, splicing RNAs, lincRNAs/antisense transcripts (AS) and other unknown RNAs. Retro-
transposons, belonging to LINEs and SINEs families, were also screened. We iden-
tified 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 per-
formed for the first time on JMML CD34+ bone marrow derived cells, showed a broad genomic hypermethylation both in pre- and post-AZA samples com-
pared to HD, suggesting a patient-specific AZA-effect. Transcription and trans-
lation processes of coding and non-coding genes could be deregulated in mul-
tiple ways, due to heterogeneity of sequences involved in CpG islands hyper-
methylation. Moreover, due to their known ability to insert random mutations in

the genome, retrotransposons should be 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

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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 (Cremer 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 cyto-
genetics.

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/high very high n=22) including 42 patients with isolated del(5q) or one additional cytogenetic abnor-
mality. The majority of analyses were performed in patients receiving lenalido-
amide or azacitidine (n=29 and n=22 patients), or in patients receiving chemotherapies/and or allogeneic transplantation or growth factors (n=10). Stan-
dardized FCM (lyse-stain-wash) and cytogentic/FISH procedures were per-
formed according to ELN guidelines at the TU of Dresden, VUMC of Amster-
dam, UH of Guadalajara and UH of Bristol. Cytogenetics/FISH analysis was con-
sidered the gold standard of all the applied FCM-scores, propagated by the ELNet MDS working group. Additionally, hematological improvement of the erythroid lineage (H-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) was considered (80% and 85%) or only MDS treated with Lenalidomide were evaluated separately (sensitivity: 98% and 100%; specificity: 85% and 75%). The use of the Ogata-score con-
sidering 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 specificity (83%) but a slightly impaired sensitivity (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 speci-
cific (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 dis-
played the del(5q)-FCM-score (p=0.0019) and Ogata-score (p=0.0092), respectively. Eval-
uating 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 OS for MDS fulfills two response criteria vs none, showing 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 estab-
lished FCM-scores allowed for an at least similar correctness of response pre-
diction. 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 back-
ground 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 ALLOGENEIC 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 allo-SCT is a potent prognostic tool. However, mutational profile at relapse after allo-SCT has not been wide explored.

**Aims:** In this study, we evaluate mutational profile at post-Allo-SCT relapse in MDS patients to determine if pre-Allo-SCT mutations are still present at relapse, so we could eventually monitor them as minimal residual disease (MRD) after allo-SCT.

**Methods:** From a retrospective cohort of 115 patients, we selected those who relapsed post-Allo-SCT (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-Allo-SCT samples to identify genetic mutations and we checked the presence of those mutations in samples at relapse. Six patients were discarded because lack of pre-Allo-SCT 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 achieved was 4570 reads (range 2401-8573). 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 (6), RAEB-1 (4), RAEB-2 (4), dysplasia associated AML (2) and RCMD (2). They relapse post-Allo-SCT 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 1 (8%) (Table 1). In 10 out of 12 evaluated patients, we found same genetic mutations at relapse compared with pre-Allo-SCT sample (Table 1). In addition, mutational pattern was similar for all patients except for one in which dominate mutation at relapse was SRSF2 present in 14% of cells pre-Allo and in 3% at relapse) instead of TET2 (51% pre-Allo-SCT and 0.6% at relapse). In 2 patients, pre-Allo-SCT mutations were not detected at relapse (Patient 8: BCOR 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-Allo-SCT. 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-Allo-SCT samples. In relapse samples, mutations were present in similar percentage in CD34 positive cells compared to CD34 depleted bone marrow (KRAS 0.63% and 2.23%, IDH2 1.6% and 1.45% respectively).

**Table 1.**

<table>
<thead>
<tr>
<th><strong>mutation</strong></th>
<th><strong>pre-Allo-SCT</strong></th>
<th><strong>relapse</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>0.63%</td>
<td>2.23%</td>
</tr>
<tr>
<td>IDH2</td>
<td>1.6%</td>
<td>1.45%</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** Post-Allo-SCT relapsing MDS show same genetic mutations found in pre-Allo-SCT evaluation, so they would potentially be used to confirm clonality and probably MRD assessment after allo-SCT 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 MDS CONTEXT.**

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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 in pts with MDS demonstrated a response rate of 78%; overall; 62% in pts following hypomethylating agent (HMA) failure and 85% in HMA naive pts (Navada et al ASH 2016).

**Aims:** To investigate the in vitro effects of RIG combined with AZA or vorinostat (VOR) on epigenetic and stem cell pathways on two cell lines: AML (BW90), MDS (MDS-L) and on pb bone marrow samples.

**Methods:** We investigated the in vitro effects of RIG combined with AZA or vorinostat (VOR) on two cell lines: AML (BW90), MDS (MDS-L) and on pb bone marrow samples. Treatment with RIG alone altered global histone post-translational modifications (PTMs) including methylation (H3K4me3, H3K4me2, H3K27me3, H3K27me2) 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 or VOR in sequential treatment (AZA/RIG, RIG/AZA, VOR/RIG or RIG/VOR) altered DNA methyltransferases (DNMT1, 3a and 3b), the class I, II and IV histone deacetylases (HDACs), and chromatin remodeler (KDM2a, SET1, JMJD3 and LRDW1) transcript levels in a cell line specific context. Sequential treatment of RIG with AZA or VOR demonstrated differential expression of the association (Pol II) with the association (Pol II) with the association (Pol II) with allele specific hypermethylated marks (H3K4me3 and H3K4me2) in both cell lines. An overall decrease in association of Pol II/H3K4me2 was observed with the combinations (AZA/RIG, VOR/RIG or vice versa) in MDS-L and BW-90, 10-33% (ANOVA, p<0.0006), 9-20% (ANOVA, p=0.0004), respectively. Significant differences were observed in pre-AlloSCT samples to identify genetic mutations and we checked the presence of those mutations in samples at relapse.

**Results:** Treatment with RIG alone altered global histone post-translational modifications (PTMs) including methylation (H3K4me3, H3K4me2, H3K27me3, H3K27me2) 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 or VOR in sequential treatment (AZA/RIG, RIG/AZA, VOR/RIG or RIG/VOR) altered DNA methyltransferases (DNMT1, 3a and 3b), the class I, II and IV histone deacetylases (HDACs), and chromatin remodeler (KDM2a, SET1, JMJD3 and LRDW1) transcript levels in a cell line specific context. Sequential treatment of RIG with AZA or VOR demonstrated differential expression of the association (Pol II) with the association (Pol II) with the association (Pol II) with allele specific hypermethylated marks (H3K4me3 and H3K4me2) in both cell lines. An overall decrease in association of Pol II/H3K4me2 was observed with the combinations (AZA/RIG, VOR/RIG or vice versa) in MDS-L and BW-90, 10-33% (ANOVA, p<0.0006), 9-20% (ANOVA, p=0.0004), respectively. Significant differences were observed in pre-AlloSCT samples to identify genetic mutations and we checked the presence of those mutations in samples at relapse. Six patients were discarded because lack of pre-Allo-SCT 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 achieved was 4570 reads (range 2401-8573). 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 (6), RAEB-1 (4), RAEB-2 (4), dysplasia associated AML (2) and RCMD (2). They relapse post-Allo-SCT 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 1 (8%) (Table 1). In 10 out of 12 evaluated patients, we found same genetic mutations at relapse compared with pre-Allo-SCT sample (Table 1). In addition, mutational pattern was similar for all patients except for one in which dominate mutation at relapse was SRSF2 present in 14% of cells pre-Allo and in 3% at relapse) instead of TET2 (51% pre-Allo-SCT and 0.6% at relapse). In 2 patients, pre-Allo-SCT mutations were not detected at relapse (Patient 8: BCOR 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-Allo-SCT. 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-Allo-SCT samples. In relapse samples, mutations were present in similar percentage in CD34 positive cells compared to CD34 depleted bone marrow (KRAS 0.63% and 2.23%, IDH2 1.6% and 1.45% respectively).

**Table 1.**

<table>
<thead>
<tr>
<th><strong>mutation</strong></th>
<th><strong>pre-Allo-SCT</strong></th>
<th><strong>relapse</strong></th>
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<tr>
<td>KRAS</td>
<td>0.63%</td>
<td>2.23%</td>
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<tr>
<td>IDH2</td>
<td>1.6%</td>
<td>1.45%</td>
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**Summary/Conclusions:** Post-Allo-SCT relapsing MDS show same genetic mutations found in pre-Allo-SCT evaluation, so they would potentially be used to confirm clonality and probably MRD assessment after Allo-SCT in the near future. CD34 selection does not provide additional sensitivity to whole bone marrow cellularity sample.
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, RRM1 and RRM2 by quantitative PCR. Corresponding proteins were evaluated in the cell lines. One patient 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 600x10^5 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 quantified by ELISA assay (Global DNA Methylation LINE-1 kit ActiveMotif, CA, USA). Results: UCK1 and UCK2 expression were evaluated prospectively in 18 IPSS high risk MDS patients treated with azacitidine 75mg/m2/day 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 RhQeQ analysis using DESeq.

Results: SKM1-R cells did not express UCK1, hCNT3, RRM1 and RRM2. Corresponding proteins were also not expressed. A reduction of apoptosis was observed in UCK1-silenced SKM1-S after azacitidine 0.1 μM treatment: 35.5%±0.57% Annexine V-positive cells versus 25%±0.35% (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%±0.85% Annexin V-positive cells versus 21%±0.35% (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, RRM1, and RRM2 and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R suggesting to be the determinant of the induced resistance 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, RRM1 and RRM2 and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R suggesting to be the determinant of the induced resistance to azacitidine. RNAseq analysis for UCK1 and UCK2 did not find any differences between responder and non-responder 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 activity (Frank 2015). TIN2F (14q12) is encoding for TIN2, the central component of the shelterin telomeric complex which interacts with other members of the complex (TRF1,TRF2 and TPP1), thus contributing to telomere length regulation and structural integrity (Frank 2015). About thirty TIN2F 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 (Ambrosi 2012). All mutations were missense and heterozygous, clustering in exon 6 encoding for a highly conserved segment at 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 68-year-old man with a multisystem disorder, i.e. psoriasis, 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 hTERT by qPCR. For the expression of TIN2F and the corresponding protein, RT-PCR and Western Blotting were performed on peripheral blood mononuclear cells (PBMCs) isolated from healthy donors and patients. Mutations were detected by Sanger sequencing and identified by Sanger sequencing, respectively.

Results: A 68-year-old man with AA was analyzed with and without panel. The expression of TIN2F and the corresponding protein, RT-PCR and Western Blotting were performed on peripheral blood mononuclear cells (PBMCs) isolated from healthy donors and patients. Mutations were detected by Sanger sequencing and identified by Sanger sequencing, respectively.

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 activity 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.
E1175
FUNCTIONAL EXPRESSION OF TIM-3 AND CLINICAL SIGNIFICANCE OF PLASMA GALACTEIN-9 LEVELS IN MYELODYSPLASTIC SYNDROMES

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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 leukemia 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 PBMCs obtained from AL-MDS patients. Soluble extracellular Ki-67 expression in F-36P cells, suggesting that Tim-3 signaling 3 induction was abrogated by adding the TGF-β receptor I kinase inhibitor dorsomorphin (DM). 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 and 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 who failed to meet the IPSS criteria for MDS. 2) Intracellular Ki-67 expression in F-36P cells co-cultured with the culture supernatant of human stromal cells and the MDS-related cytokine transforming growth factor-β1 (TGF-β1). Tim-3 cell-surface protein and mRNA expression in MDS cell lines was induced by co-culture with TGF-β1. The Tim-3 induction was abrogated by adding the TGF-β receptor I kinase inhibitor SB431542. 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 gene expression analysis (GSEA) showed enrichment of pathways involved in cell proliferation and antiproliferative responses in Tim-3+ cells. 4) The blockade by anti-Tim-3 antibody decreased intracellular Ki-67 expression in F-36P cells. 5) Co-immunoprecipitation experiments showed that the new Tim-3-gal-9 binding with TRF2 in vitro.

Summary/Conclusions: A new TIMF2 gene variant at exon 2, c.254A>G p.H85G, was identified in the proband and in two 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 SNP's profile and WES identified an additional somatic mutation in TLR1 gene (c. 1859G>A. p.R620Q). Co-immunoprecipitation experiments showed that the new TIMF2 mutation reduced TIM2 binding with TRF2 in vitro.

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 selecting the proper therapeutic options. Therefore, identification of risk stratifying paradigms, 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 (Papenau et al., Blood, 2013) and/or structural variants detected by single nucleotide polymorphism (SNP) arrays (Cara et al., Blood, 2015) have shown a potential for score criteria in the diagnosis of MDS. Recent reports of the presence such genome aberrations in disease free individuals makes this approach problematic (Ghosh 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 (Cargo 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, 70 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 TruSight on a MSq platform (Illumina, USA) was used to screen mutational hotspots in 5 cancer-related genes relevant in myeloid malignancy. Genes were variances 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 genome 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, the rest as MDS positive. Genotype variances were detected in all but 7 (4%). The latter are void of gene mutations. We observed driver mutations as reported in myelomas 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 TruSight mieloid panel. In order of frequency these are TET2, SRSF2, ASXL1, CUX1, DNM3A, RUNX1, BCR1 and HRA5, 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 driver variances in samples with and without disease although reports of frequent driver mutations (Papenau et al., Blood, 2015) have shown a potential for score criteria in the diagnosis of MDS. Recent reports of the presence such genome aberrations in disease free individuals makes this approach problematic (Ghosh 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 (Cargo 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.

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 if we compare the two groups by variance, 9 variances are exclusively associated with MDS positive disease.

E1177
SUPPRESSION OF DNA METHYLTRANSFERASE ENZYMES BY A NOVEL HYPMETHYLATING AGENT, SG-1027, IS AUGMENTED IN DECITABINE-RESISTANT CELL LINES

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Background: We established azacitidine- and decitabine-resistant cell lines, MOLM/aza-1 and MOLM/dec-5 from MOLM-13, an acute myeloid leukemia cell line (OncoCell in press). DNA methyltransferase (DNMT) 3B was upregulated in the resistant cell lines.

Aims: We tried to find out clues to overcome the resistance to hypomethylating agent (HMA).
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 (DNMT1, 3A, and 3B) were assayed before and after treatment of each HMA. Proteosomal degradation and activation of p-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 IC_{50} 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 Nanomycin 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) of mitochondrial TRX (siTRX2) 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.
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 nature of PAS positivity. 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 lineages. PAS positivity was significantly higher in MDS patients than in normal and pathological controls, with a significant difference between MDS and non-erythroid controls (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 0-53) 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 differences in relation to excess blasts or multilineage dysplasia. PAS 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-RS, 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 observed between PAS score values and internuclear bridging (r=-0.23, p=0.0395). A ROC curve analysis allowed us to identify a PAS score value of ≥1 (AUC=0.697, p=0.0008) and a PAS positive erythroblast percentage of ≥1% (AUC=0.674, p=0.0034) as optimal cutoff to discriminate MDS patients from non-erythroid 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 -globin synthesis, but higher than that of defective hemoglobinization, nuclear lobulation, multinucleation, cytoplasmic fraying, pyknosis, and internuclear bridging. Integrating 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 suspected 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 MYELODYSPLASIC SYNDROMES (MDS)

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Background: DAR was randomized in 400 patients in 3 arms: PBO, DAR, or darbepoetin alfa Q3W for 24 weeks, with intermediate-2 (INT-2) risk patients stratified at enrollment. The primary endpoint was transfusion rate at 48 weeks. The main secondary endpoints were HI-E rate at 48 weeks and incremental transfusion-free survival (TFS) rate with Q2W dosing. Major secondary endpoints were RBC transfusion, Hb response, and safety.

Methods: Patients were randomized to PBO, or darbepoetin alfa Q3W or Q2W at the beginning of the treatment period. Dose was increased to 200 µg/week if the target Hb level was >12 g/dL, and decreased if the Hb increased by >1.5 g/dL in 3 weeks. Key endpoints were transfusion incidence and Hb-E in both PBO and DAR arms.

Results: At study entry, 147 patients were randomized to PBO and 147 to DAR. At 48 weeks, 34 patients (23%) in the PBO arm had transfused compared to 14 (10%) in the DAR arm, p=0.046. In the DAR arm, 31 patients (21%) had an Hb-E rate ≥50% compared to 19 (13%) in the PBO arm, p=0.032. The incremental TFS rate was 53% in the DAR arm compared to 42% in the PBO arm, p=0.031. No significant differences were observed in transfusion incidence or TFS rate between DAR Q3W and Q2W.

Conclusions: This study demonstrates that darbepoetin alfa Q2W is non-inferior to Q3W in terms of transfusion incidence and TFS rate and offers the clinical benefit of more frequent dosing and improved Hb-E rate at 48 weeks.

E1179

EVALUATING ERYTHROBLAST PAS POSITIVITY IN THE DIAGNOSTIC APPROACH OF MYELODYSPLASTIC SYNDROME

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Background: PAS positivity is included into the morphological scoring system we have previously defined (Leukemia 2015;29:66-75).

Aims: PAS positivity was included into the morphological scoring system we have previously defined (Leukemia 2015;29:66-75).

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 their 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 lineages. PAS positivity was significantly higher in MDS patients than in normal and pathological controls, with a significant difference between MDS and non-erythroid controls (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 0-53) 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 differences in relation to excess blasts or multilineage dysplasia. PAS 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-RS, 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 observed between PAS score values and internuclear bridging (r=-0.23, p=0.0395). A ROC curve analysis allowed us to identify a PAS score value of ≥1 (AUC=0.697, p=0.0008) and a PAS positive erythroblast percentage of ≥1% (AUC=0.674, p=0.0034) as optimal cutoff to discriminate MDS patients from non-erythroid 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 -globin synthesis, but higher than that of defective hemoglobinization, nuclear lobulation, multinucleation, cytoplasmic fraying, pyknosis, and internuclear bridging. Integrating 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 suspected MDS, especially if there is only unilineal dysplasia without ring sideroblasts or excess blasts.

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: LEN recruited 223 patients with transfusion-dependent MDS and 5q deletion to the 48-week maintenance phase of the phase 3 study. MDS with transfusion independence due to ESAs were randomized 1:1 to placebo or LEN 10 mg daily. The primary endpoint was transfusion independence at 48 weeks. Key secondary endpoints were Hb response and TFS rate at 48 weeks.

Methods: Patients were randomized 1:1 to placebo or LEN 10 mg daily. The primary endpoint was transfusion independence at 48 weeks. Key secondary endpoints were Hb response and TFS rate at 48 weeks.

Conclusions: LEN was associated with greater transfusion independence, Hb response, and TFS rate compared to placebo at 48 weeks. These results provide a rationale for further investigation of LEN in this patient population.
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 randomized 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 Sintra-Rev 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 cytogenetic responses early after treatment with an adequate safety profile in

Summary/Conclusions:

SAE were not related with the drug of the study (LEN/Placebo).

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Background: Myelodysplastic syndrome (MDS) are the most frequent hematological malignancy in elderly patients. The impact of MDS burden on overall mortality remains controversial, moreover, after the incorporation of hypomethylating 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) of which 58% (56%) were males. MDS was primary in 574 (54%) 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 categories 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), 10% at 24 months (14-19) and 25% at 60 months (95%CI 22-28) of mortality and by 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 demonstrated 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.

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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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 14/38 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 5%. Seven patients AE were reported in 5 patients: vestibular neuritis, congestive heart failure, polyarthitis, 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.

E1183

PROSPECTIVE STUDY OF FLOW CYTOMETRY OF BONE MARROW IN 105 CONSECUTIVE PATIENTS WITH CYTOPENIA AND SUSPICION OF MYELODYSLASTIC SYNDROME: STRONG CORRELATION WITH RISK OF AML-EVOLUTION AND SURVIVAL

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Background: Diagnosis of myelodysplastic syndromes (MDS) remains a challenge, specially in patients with scant displastic morphology features and/or in the absence of cytogenetic changes. Multiparametric flow cytometry (MFC) findings have been recognized as a co-criterion for the diagnosis of MDS and we have also demonstrated prognostic value in some studies. Nevertheless, this diagnostic tool is not fully implemented for the study of MDS in many centers and data from real life out of investigational studies are few.

Aims: To prospectively assess the value of MFC in the diagnosis of MDS in our center and correlate its findings to the clinical outcome of patients in terms of overall survival, transfusional needings, risk of hospitalization and evolution to acute leukemia.

Methods: We studied bone marrow samples from 105 consecutive patients submitted to our hospital between January 2013 and April 2015 because of one or more cytopenia and suspicion of MDS. Cytomorphology of every sample...
was evaluated by at least two morphology experts and a consensus diagnosis of MDS-confirmed, MDS-suspected or MDS-excluded was emitted. MFC was performed applying at least five-colour 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-, 8q-, 20q- and del(7q) were performed in all cases.

Results: Median age of the patients was 73.5 y/o. Patients presented with anaemia in 88% (84%), neutropenia in 36% (34%) and thrombocytopenia in 49% (47%). Cytomorphology was reported as MDS-confirmed (60 pts), MDS-excluded (22) or MDS-suspected (23). MDS subtypes were Multilineage Dysplasia (23), Unilineage Dysplasia with Ring Sideroblasts (9), del(5q) Syndrome (3) and Unclassified (2). 4 pts being diagnosed of CMLML. MFC score was MDS-suggestive in 56 pts, MDS-not suggestive (36) and in 13 cases its use was precluded because of morphology findings. Considering cytomorphology as gold standard, 56.9% of patients with MDS-confirmed MFC score were expected but MS-suspected but 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 to significant association with del7q 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 findings 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 diagnostic 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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¹Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, ²Xcenda, Palm Harbor, United States

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-9/10 code (ICD-9 code: 234.73; 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 a primary diagnosis of MDS or MDS-related treatment (ie, MDS chemotherapy as defined by NCCN MDS Guidelines v2.2017 or MDS-directed supportive care which included hydroxyurea, erythroid-stimulating-colony-stimulating-growth factors and prophylactic platelet transfusions) and pharmacy claims for MDS treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPMM) costs adjusted to 2015 US dollars and reported as mean (standard deviation [SD]). Patients with a capitated payment were excluded from analysis. Costs were reported as mean (standard deviation [SD]). Patients with a capitated payment were excluded from analysis. Costs were reported as mean (standard deviation [SD]). Patients with a capitated payment were excluded from analysis.

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 ≥1 MDS-related, 1 and outpatient visit over the follow-up period, and 56.9% had ≥1 physician office visit (91.9%) and other outpatient visits (99.5%). Over the follow-up period, the mean PPPMM cost was $17,361 (SD: $19,747) (Table 1) and was higher in Year 1 than in Year 2 ($17,337 [SD: $19,696] vs $12,076 [SD: $14,135]). The majority of costs overall were for MDS-related medical services ($10,327 PPPM, SD: $11,050). Between Years 1 and 2, MDS-related medical PPPM costs decreased from $10,557 (SD: $11,164) to $6,530 (SD: $7,406) while non-MDS-related medical PPPM costs remained fairly constant in both years. Chemotherapy and supportive care medical services were the main drivers of MDS-related medical costs, also decreasing from Year 1 to Year 2. Non-MDS-related costs accounted for a smaller portion of the overall medical PPPM costs ($6,124 [SD: $15,158]); and remained relatively similar in Years 1 and 2.

Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean PPPM Cost (SD)</th>
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<tr>
<td>Year 1</td>
<td>$17,337 ($19,696)</td>
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<tr>
<td>Year 2</td>
<td>$12,076 ($14,135)</td>
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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 a peripheral cytopenia, and can be another target for therapeutic intervention.

Aims: To explore the role of intravenous immunoglobulin (IVIG) as a treatment for immune-related cytopenia 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 a 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 clonal t(11;19) and 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
(3), seronegative arthritis (2), connectivitis (2). One patient with hypoplastic MDS had LGL liver involvement. Coombs test was positive in 4/16 hemolysis cases. From Jan-10 to Jan-17, IVIG was administered at a dose of 500mg/kg once per week, in cycles of 1 to 4 weeks. The ORR was 75% (15/20); all patients showed an erythroid hematological improvement (HI) (100%). Platelets and neutrophil HI was seen in 50% and 80% of responsive cases, respectively. HLR occurred in 13/16 (81%: 4 CR and 9 PR). Median number of cycles and duration of treatment was 11 and 12 months (mo), respectively. The HLR-CR was stable in 7 patients; 4 relapsed from HLR but subsequently responded by shortening the intervals between administrations of IVIG; 2 were secondary refractory. Eventually, 6 responders became refractory to IVIG. Response was more durable with continuous rather than sporadic dosing. Median time to response was 1 mo. Median duration of response was 39 mo. Corticosteroids were discontinued in 5/10 patients and reduced in 5/10. Adverse events were: 1 palpitations (G1); 1 hypertension (G1). Responders had lower platelet counts (p<0.05), but no other clinical differences compared to non-responders. However, the 5-year OS rate was higher in the responders to IVIG: 53% compared to 30% (p=0.08).

Table 1.

Summary/Conclusions: Treatment with IVIG of C± hemolytic anemia and pancytopenia associated with T-cell immune-clones and MDS was safe and yielded high rates of durable response on all lineages and on hemolysis. Transfusion independency and reduction/discontinuation of corticosteroids for chronic hemolysis 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 progression to a lower IPSS 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 is more robust and is able to distinguish the survival outcomes of patients 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 different 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, 0.61 vs 0.57) as well as in the independent cohort including pre-treated patients (C-statistic, 0.58 vs 0.54).

Conclusion: 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.

E1187

PROGNOSTIC AND THERAPEUTIC IMPLICATIONS OF SIGNIFICANT MARROW FIBROSIS IN COMBINATION WITH P53 OVER-EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME: A SINGLE CENTRE STUDY

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Background: Myelodysplastic syndromes (MDS) are defined in the WHO 2016 classification as a group of clonal bone marrow neoplasms characterized by ineffective hematopoiesis, morphologic dysplasia in hematopoietic cells and peripheral cytopenia(s). They present as a diverse phenotype with some patients requiring merely observation while others require more intensive management due to significant marrow failure and the risk of development of acute leukemia. The presence of significant marrow fibrosis has previously been shown to be a poor prognostic factor in patients with MDS, with reduced overall survival. Significant marrow fibrosis has also been associated with both the presence of the TP53 gene mutation and with p53 over expression, which is a known adverse prognostic risk factor in MDS patients.

Aims: To assess the presence of p53 expression in patients with moderate to severe marrow fibrosis (grade 2-3), observe its effect on overall survival in patients with marrow fibrosis, and determine whether the use of azacitadine had any impact on survival.

Methods: We conducted a retrospective study utilizing a hospital database of 247 patients with MDS diagnosed in a single center between 2000 and 2014. Of these patients, 200 had bone marrow trephine samples adequate for reticulin stain analysis, which was completed using the European consensus on grading bone marrow fibrosis (grades 0-3). P53 expression was examined using immunohistochemistry staining according to the modified quick scoring system. We then looked for an association between degree of marrow fibrosis and p53 expression. In patients with significant marrow fibrosis and p53 expression, we examined overall survival and response to treatment with azacitadine.
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 azacitidine, though most of significant fibrosis and p53 expression had a significantly increased overall survival compared with those who did not receive azacitidine (4 months versus 1 month, p=0.002). Azacitidine 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 azacitidine had a significant increase in median survival. Although the numbers of patients who received azacitidine (23% in the azacitidine data) suggests that patients with fibrosis may benefit from the use of azacitidine and larger, randomized studies should be considered to study this further.

References

E1188
FACS PURIFICATION OF BLAST CELLS IN MDS IMPROVES THE FISH 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 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 lab, FISH was historically performed on full marrow aspirates (Full Sample); 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 2018 in which a FISH panel workup for a suspected diagnosis of MDS presented with 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.

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 standard therapy (2), 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 counted as CR, PR or no response.

Results: 22nd Congress of the European Hematology Association
E1189
COUNTING BONE MARROW BLASTS AS A PERCENTAGE OF NON-ERYTHROID CELLS PROVIDES SUPERIOR RISK STRATIFICATION FOR MDS PATIENTS WITH ERYTHROID PREDOMINANCE
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Background: Patients with erythropoietic predominance (50% erithroblasts, MDS-erythroid) compose a significant proportion of patients with MDS. The erythroid/myeloid subtype was divided from the AML category into MDS-erythroid by the 2016 WHO classification of myeloid neoplasms. At that time, there was no consensus on a more appropriate way of enumerating bone marrow (BM) blasts from TNCs or NECs in MDS-erythroid patients.

Aims: To clarify these questions, 1283 MDS patients were retrospectively analyzed in our center.

Methods: MDS-erythroid was observed in 27.0% of patients (346/1283), and these patients had similar clinico-pathological features and overall survival, with 103 cases of MDS with <50% ENCS (Erythroblasts in Non-Erythroid Cells) blasts.

Results: By calculating the percentage of BM blasts from NECs, 73 of 200 patients (36.5%) with MDS-erythroid who were diagnosed within WHO subtypes without excess blasts (EB) were moved into higher-risk categories and showed shorter OS than those who remained in the initial categories (P=0.041). Recalculating the International Prognostic Scoring System-Revised (IPSS-R) by enumerating blasts from NECs, 40 of 168 (23.8%) MDS-erythroid patients with relatively lower risk were re-classified as higher-risk and had significantly poorer survival than those who remained in the lower-risk category (P=0.030). This was especially true for the intermediate risk group that was stratified by IPSS-R (unchanged patients vs shifted patients, P=0.007). However, the impact of enumerating BM blasts from NECs on classification and prognostication was not evident in all MDS patients.

Summary/Conclusions: In conclusion, our results suggested that enumerating the percentage of BM blasts from NECs significantly improved the prognostic assessment of MDS-erythroid, especially for patients within the intermediate risk group stratified by IPSS-R.
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 lines 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).

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 danazol for prolonged periods, when tolerated. Response to danazol is also potentially associated to a survival advantage, although these data should be confirmed by larger prospective studies.

E1191
SURVIVAL OUTCOMES 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: MDS is composed of multiple and rare hematological stem-cell disorders, resulting in cytopenias and disease-related complications and deaths. There are no robust trial data comparing the available treatment options for HR-MDS patients; and of the approved drugs, only azacitidine has demonstrated a statistically significant, but modest clinical impact on overall survival (OS).

Aims: We evaluated first-line treatment (1LT) choice and survival outcomes in a US cohort of HR-MDS patients engaged in routine care.

Methods: Newly diagnosed HR-MDS patients who were ≥18 years old and who had initiated 1LT were retrospectively identified from Optum, a large US claims database, between 1/1/2008 and 10/31/2015. HR status was based on prior treatment for MDS or chronic myelomonocytic leukemia (cMML) and the presence of other primary cancer, or receipt ≥1 HR-MDS ICD-9/10 code (ICD-9 code: 238.73; ICD-10 codes: D46.20, D46.21, D46.22), or ≥2 outpatient claims with ICD coding: ≥1 inpatient claim with an HR-MDS ICD-9/10 code (ICD-9 code: 284.5). The first MDS claim served as the index diagnosis (E1190). The primary outcome evaluated was overall survival (OS), with secondary endpoints of progression-free survival (PFS), transplant-free OS (TFO-S), and event-free survival (EFS).

Results: 209 newly diagnosed HR-MDS patients initiating 1LT MDS-Tx were identified; mean age was 73 years (standard deviation [SD]: 10.1) and 61.2% were male. In the 12 months prior to diagnosis, 27.3% of patients used MDS-directed supportive care (ie, colony stimulating-, erythropoietic-, or thrombopoietic growth factors; RBC or PLT transfusions; or hydroxyurea). 1LT with hypomethylating agents (HMAs) predominated in 89.5% of patients (azacitidine, 68.9% and decitabine, 20.6%); 8.6% of patients received an immunomodulator monotherapy; and 8.6% of patients underwent SCT during follow-up. Of the 169 treated HR-MDS patients with ≥60 days of follow-up on 1LT, 51% achieved transfusion independence. For all treated HR-MDS patients, median PFS and 2-year follow-up were 12.5 months (95% confidence interval [CI]: 9.1, 14.9) and 27.0%, respectively. OS rate at 2 years was 59.1%. Patients who achieved transfusion independence had a higher rate of 2-year OS (65.2% vs 53.8%) and PFS (36.3% vs 25.7%), but neither were statistically significant.

Table 1.

Table 1.

Figure 1.

Summary/Conclusions: Survival outcomes in routine clinical care were higher than reported in clinical trials, specifically in HR-MDS trials with azacitidine. Among patients able to achieve transfusion independence, a trend toward increased 2-year PFS and OS rates was observed, although statistical significance was not reached. Characteristics that contribute to variations in PFS and OS outcomes within the HR-MDS population need further investigation.

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 128:112)

Aims: To confirm pharmacokinetic (PK) and pharmacodynamic (PD) comparability 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 (CML) 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 or 5 days of ASTX727, followed by a cross-over to the other Cycle

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 danazol for prolonged periods, when tolerated. Response to danazol is also potentially associated to a survival advantage, although these data should be confirmed by larger prospective studies.
2. Cycles 3 forward were with ASTX7727. PD were assessed with LINE-1 methylation measured on white blood cells at baseline at days 8, 15, 21 and 28 in cycles 1 and 2. Full PK assessments of ASTX7727 were performed on Days 1, 2 and 5 with sparse sampling on Days 3 and 4 and on Day 1 of IV-DAC. Modeling of 5 day exposures of ASTX7727 and IV-DAC was created for each patient. Safety and clinical response were assessed on all patients.

Results: Among the 50 randomized, 50 had matched PK and 46 had matched PD sample sets for the first 2 cycles. No significant differences were seen when comparing the randomized sequences for any parameters, so all assessments comparing ASTX7727 and IV-DAC were performed independent of sequence. The geometric mean maximum demethylation was 9.9% for ASTX7727 vs 9.0% for IV-DAC (Ratio of geometric means [GMR] 1.099, 95% CI: 0.806-1.000; p=0.34). The geometric mean AUC for IV-DAC was 161 ng/h/mL. The 5 day total geometric mean of the AUC (ng/mL) was 769 for ASTX7727 and 805 for IV-DAC ([Ratio of geometric means [GMR] 0.96, with a lower limit of 95% CI of 0.806]. Decitabine C max was higher for IV-DAC (189 ng/mL) than after ASTX7727 (210 ng/mL) and 5 day AUC (112 ng/mL). The Day 1 adverse events regardless of grade or causality were febrile neutropenia 34%, neutropenia 28%, thrombocytopenia 16%, fatigue 16%, and hypomagnesaemia 18%. There were no reported GI adverse events greater than Grade 2 with ASTX7727 regardless of relationship to treatment.

Summary/Conclusions: Fixed dose oral administration of 35mg decitabine and 100mg E7727 (ASTX7727) emulates the AUC of IV-DAC over the 5 day treatment cycle and induces a similar degree of demethylation of LINE-1 sequences in blood cells compared to IV decitabine at 20mg/m 2 dose. The preliminary safety profile is similar to what has been reported for IV-DAC and no significant differences were seen when comparing the randomized sequences for any parameters, so all assessments comparing ASTX7727 and IV-DAC were performed independent of sequence. 

E1193 FACTORS PREDICTIVE FOR INFECTION IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES, CHRONIC MYELOMONOCYTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA TREATED WITH AZACITIDINE

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Background: Hypomethylating agents (HMAs) are the current standard treatment of higher-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CML) and older acute myeloid leukemia (AML) patients. Severe infectious complications are common during HMAs therapy and have been recognized as one of the most important reasons of morbidity and mortality in MDS, CML and AML patients especially at the beginning of treatment.

Aims: To evaluate the incidence of and predisposing risk factors for infections during the first three azacitidine (AZA) cycles treatment

Methods: In this retrospective study we analyzed 282 consecutive patients with higher-risk MDS (174), CML (34) and AML with bone marrow blasts <30% (74) treated with azacitidine in 10 Polish hematologic centers between the years 2009-2016. Patients who did not complete three AZA cycles and also did not experience infection were excluded (50 cases).

Results: Median age was 68 (28-93). Median number of AZA cycles was 6 (1-24). Infectious episodes occurring during the first three courses of AZA were reported in 94 out of 232 (40%) eligible patients - in 32% of MDS, 30% of CMML and 63% of AML patients (p<0.05). Among patients with infection, most of them had their first infection episode during the first cycle -53%, during the second cycle -29% and during the third AZA cycle -18%. It was found that low neutrophil count (<1.1x109/L), low lymphocyte count (1,1x109/L vs 1,3x109/L), low platelet count (37x109/L vs 63x109/L), high blood ferritin level (1101ng/ml vs 569 ng/ml), low albumin concentration (3,6 g/dl vs 4,0 g/dl), high bone marrow blasts percentage (15% vs 12%), higher IIHS, IPSS-R and WPS score, red blood cell transfusion dependency and worse WHO performance status predicted infectious episodes. The most important predictive factors for infection (p<0.05). Factors like age, gender, hemoglobin concentration, iron, immunoglobulin and creatinine blood level, time from diagnosis to beginning of AZA treatment, IPSS and IPSS-R cytogenetics, coexistence of diabetes mellitus, heart failure, chronic obstructive pulmonary disease, second cancer, autoimmune disease and corticosteroids treatment had a negative influence. The most important predictive factors for infection 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 score 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 taking 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 at diagnosis) were included with a median follow-up duration of 70.2 months. 15.7% of the population had an IPSS score ≥3 and 12.4% of the population had an IPSS-R score ≥4. In 41.5% no cytogenetic information was available. MDS-RRS, 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. 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 and representative population-based data on overall survival and treatment of patients with MDS, IPSS, IPSS-R score and MDS subtype according to WHO 2016 classification. Further, the leukemia free survival (LFS), the initial treatment and the duration of first-line treatment were analyzed.
Sustainable, 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 improve 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/μL, the average was 20x10^3/μL and the maximum value was 38x10^3/μL. 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/μL 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 is over 75% after two years from the beginning therapy in the responder patients (Figure 2). Adverse events recorded were as follows: moderate (grade 1 and 2) increase in transaminases in 4 cases (with reduction of danazol to 400mg/day in 2 of these); severe (grade 3) (with subsequently drug suspension); severe renal failure in 1 case (the drug was stopped); moderate (grade 1 and 2) increase of serum creatinine in 6 case (with reduction of danazol to 400mg/day in 2 of these); reversible cutaneous rush 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. 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/μL, the average was 20x10^3/μL and the maximum value was 38x10^3/μL. 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).

**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 loss 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.

**J. Bell** et al.,

**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: ≥1 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.

**Figure 1.** First-line treatment patterns in HR-MDS patients

**Figure 2.** Progression free survival

**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%, IQR: 67.2, 75.3 vs 55.0, 67.2, respectively, p=0.0007). 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=43), 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 a second HMA treatment with a median duration of 1.9 months (IQR: 1.0, 6.1) for azacitidine and 2.3 months (IQR: 1.0, 10.7) for decitabine, p=0.005. A greater proportion of decitabine-treated patients received a second HSCT with a median duration of 4.2 months (IQR: 1.0, 10.7) for azacitidine and 7.9 months (IQR: 3.0, 18.0) for decitabine, p<0.001.
received supportive care with colony-stimulating factors (CSFs) (39.5% vs 28.5%) and either erythropoiesis 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 HMAAs 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 end of study duration), 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
APPRECI8: A PIPELINE FOR PRECISE VARIANT CALLING INTEGRATING 8 TOOLS
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Background: For the use of next-generation sequencing in clinical routine same platform, on a different platform and expert-based review. The same target region consisting of 19 genes was analyzed. Validation was performed by re-sequencing on the Roche 454. In all cases the same target region was sequenced on Illumina HiSeq. The second test set covered 89 MDS patients, sequenced in 2 different test sets. The first test set covered 237 MDS patients, sequenced on Illumina NextSeq. Subsequently, two independent test sets were analyzed. The first test set covered 237 MDS patients, sequenced on Illumina HiSeq. The second test set covered 89 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.

Aims: We developed a variant calling pipeline with both, high sensitivity and high PPV.

Methods: We developed appre8, a variant calling pipeline combining the output of 8 open-source variant calling tools: GATK HaploTypeCaller, Platypus, VarScan, LoFreq, FreeBayes, SNVat, Schem8 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 113 MDS patients, sequenced on Illumina NGSX3. Subsequently, two independent test sets were analyzed. The first test set covered 237 MDS patients, sequenced on Illumina HiSeq. The second test set covered 89 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 polymorphisms - 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 EFFICIENCY 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 myeloid leukemia with multilineage dysplasia with less than 30% 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 azacytidine (Vidaza®) in the dose of 75mg/m2x7 every 28 days and 4 patients were treated with decitabine (Dacogen®) in the dose of 20mg/m2x5 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% for SCT vs 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.

Figure 1.
A MULTICENTER, OPEN-LABEL, PHASE I CLINICAL STUDY: SAFETY, EFFICACY, AND PHARMACOKINETICS OF ORAL RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES

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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 period, 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 septic shock caused by urinary tract infection. In the 560mg BID arm, 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 narrow 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 high.

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.

MYELODYSPLASTIC SYNDROMES - HIGHLIGHTS NOVEL CANDIDATE PROGNOSTIC MARKERS AND DISEASE SUBGROUPS

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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, E2F2 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 E2F2 dependency in MM cells.

Aims: In this study, our aim is to defined the regulatory landscape of E2F2 in MM to better understand how E2F2 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 E2F2 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 genome. 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 genome revealed specific co-occupancy of the factors at promoters of active genes marked by H3K4me3, with a strong positive correlation between E2F2 and RNA Pol II expression (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 E2F2 and BETs to identify their individual contribution to eventual functional effect in MM. Unbiased hierarchical clustering revealed distinct regulatory axes for E2F2 and BETs, with E2F2 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 E2F2 promoter signal. We hypothesized that the presence of BETs and E2F2 in distinct regulatory axes divides active genes in MM into those that can be selectively influenced by BET inhibition or E2F2 perturbation, but not both. In line with this we have observed that dual E2F2 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.

ANALYSIS OF THE GENOMIC LANDSCAPE OF MULTIPLE MYELOMA HIGHLIGHTS NOVEL CANDIDATE PROGNOSTIC MARKERS AND DISEASE SUBGROUPS

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Background: In multiple myeloma (MM), next generation sequencing (NGS) has expanded our knowledge of genomic lesions, and highlighted a dynamic and heterogeneous composition. Despite a growing number of cases sequenced, the full potential of NGS studies has not been exploited so far.
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, BRF4, DIS3, TRAF3, SPH40, IRF4) 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), ampl(1q) (HR 2.83, 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 one, 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 ampl(1q), del(13), del(17p), 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 DATA IN PATIENTS WITH MULTIPLE MYELOMA

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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 genomic 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 hybridization-based targeted sequencing panel in order to further provide critical prognostic information at a lower cost and 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, with which the algorithm also examines clinical biomarkers. RNAseq data and expression arrays were used to understand the relevance in myeloid and lymphoid malignancies. Targeted sequencing was applied to a dataset of 126 MM patients with known copy number status as determined by conventional cytogenetics, FISH and MLPA. Our novel method detected numerous copy number changes that were beyond 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 heterogeneity of changes on del(1p) including isolated deletions of FAMM6C, CDK2NC 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 monoallelic and biallelic TP53 aberration was associated with poor survival. Overall findings in this cohort include frequent DIS3 mutations in patients with IGH translocations (t(14;16) and 2p frequently 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 and validation of changes 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.

E1203

THE MULTIPLE MYELOMA GENOME PROJECT: DEVELOPMENT OF A MOLECULAR SEGREGATION STRATEGY FOR RISK STRATIFICATION OF MULTIPLE MYELOMA


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Background: Segmenting multiple myeloma (MM) into subgroups with distinct pathogenesis and clinical behavior is critical to implement a targeted therapy approach and improve prognosis for patients. Current technologies have elucidated major translocation groups and recurrent copy number changes with varying effects on prognosis. However, minor translocation and mutational groups remain poorly described due to limited sample numbers and small datasets. The availability of multiple sets of high quality genomic data associated with clinical information, cytogenetics, and outcomes provides an opportunity to create an integrative genomic predictor using mutational, chromosomal, and gene expression alterations to develop a classification system to segment MM into therapeutically meaningful subgroups.

Aims: The Multiple Myeloma Genome Project (MGP) is a global collaborative research project to develop a data-driven genomic molecular segregation strategy for MM to inform development and deployment of clinically relevant tests that could improve diagnosis, prognosis, and treatment of patients with MM.

Methods: We have established a dataset representing 1766 MM patients for which whole exome sequencing (WES; n=1397), Whole Genome Sequencing (WGS; n=779), and expression data from RNA-seq and expression arrays (n=1059) were available. Data were derived from the Myeloma XI trial, Dana-Faber Cancer Institute/Intergroupe Francophone du Myelome, The UAMS Myeloma Institute and the Multiple Myeloma Research Foundation (IA1 – IA9). Data were investigated for genetic abnormalities following preprocessing with state of the art methods and algorithms.

Results: Our analysis is focused on data from newly-diagnosed MM patients (n=1751), which is the majority of our dataset. We have begun to integrate genomic data with various correlates. Based on our data, we have at least...
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, intrinsic resistance to venetoclax treatment observed in MM patient samples has been attributed to a low BCL-2-to-MCL-1 gene expression ratio, suggesting a central role for MCL-1 in cell survival in this context as well. NOXA functions to sequester the anti-apoptotic BCL-2 family member, MCL-1. Increased MCL-1 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 MCL-1 to treatment efficacy in MM, we investigated the ability of an MCL-1-lowering agent, namely the CDK9 inhibitor alvocidib, to potentiate the activity of venetoclax in 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 vitro 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 cell lines and primary MM cells purified from patients but not in PBMCs 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 of VCP.

Summary/Conclusions: We have elucidated emerging roles of VCP and its potential as a therapeutic target in several cancer subtypes including multiple myeloma (MM).

Summary/Conclusions: Taken together, our data suggest that the combination of alvocidib and venetoclax in the context of bortezomib resistance further, it suggests that CDK9-mediated targeting of MCL-1 may offer a clinical route to addressing intrinsic resistance in MM patients.

E1205

NOVEL COMPOUND, OSSL_325096, INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS THROUGH VCP INHIBITION

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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 vitro 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 cell lines and primary MM cells purified from patients but not in PBMCs 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 of VCP.

Summary/Conclusions: We have elucidated emerging roles of VCP and its potential as a therapeutic target in several cancer subtypes including multiple myeloma (MM).

Summary/Conclusions: Taken together, our data suggest that the combination of alvocidib and venetoclax in the context of bortezomib resistance further, it suggests that CDK9-mediated targeting of MCL-1 may offer a clinical route to addressing intrinsic resistance in MM patients.

E1206

A NOVEL PREDICTIVE MODEL COMBINING LINCRNAS AND PROTEIN CODING GENES IN MULTIPLE MYELOMA

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CODING GENES IN MULTIPLE MYELOMA

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Summary/Conclusions: We have elucidated emerging roles of VCP and its potential as a therapeutic target in several cancer subtypes including multiple myeloma (MM).

Summary/Conclusions: Taken together, our data suggest that the combination of alvocidib and venetoclax in the context of bortezomib resistance further, it suggests that CDK9-mediated targeting of MCL-1 may offer a clinical route to addressing intrinsic resistance in MM patients.
**Background:** RNA has diverse sets of regulatory functions and a recent analysis by a large 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 role in the immune dysregulated microenvironment has never been investigated. First, we observed an increase in inflamed microenvironment markers (increase in CD4+ and CD8+ cell count in favor of CD4+/population and HLA-DR expression on plasmacells and non-plasmacells) during the course of SMM. Secondly, 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 sequential samples of SMM patients. Expression of checkpoint markers was augmented in samples of patients with progressed SMM as compared to patients with stable SMM. These findings suggested that 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 VESELIC SECRETION INDUCES APOPTOSIS OF BONE MARROW STROMAL CELLS: TOWARDS SOIL-TARGETED THERAPY IN MULTIPLE MYELOMA**

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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 reconstituting immune system may be a favourable context for immunotherapies, including cellular therapy and checkpoint blockade. Autologous MM cells aimed to characterize immune composition and function and LAG3 expression in MM cells post-ASCT, to identify candidate immune checkpoint proteins for therapeutic targeting.

Methods: BM aspirates were obtained from patients with MM at 3-months post ASCT (n=28), and 6-12months post ASCT (n=41) at University College Hos- pital. Control BM aspirates were collected from healthy volunteers undergoing BM harvesting with the Anthony Nolan. Immunofluorescence surface staining was performed using antibodies to CD3, CD4, CD8, LAG-3, PD-1, HLA-DR, ICOS and the intracellular markers GzmB and Foxp-3. All p-values indicate differences from normal donors unless otherwise stated. The absolute number of post-ASCT patients with residual disease have higher numbers of cytotoxic CD4 and CD8 cells 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.

**Summary/Conclusions:** From, we observed an increase in inflamed microenviron- ment markers (increase in CD4+ and CD8+ cell count in favor of CD4+/population and HLA-DR expression on plasmacells and non-plasmacells) during the course of SMM. Secondly, 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 sequential samples of SMM patients. Expression of checkpoint markers was augmented in samples of patients with progressed SMM as compared to patients with stable SMM. These findings suggested that 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 is mediated by 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 cell adhesion method. EVs were isolated from conditioned medium of BMSCs using a Exoquick-TG (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). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). For functional analysis of candidate miRNAs, the miRNA mimics (Ambion) were transfected into BMSCs using HiPerFect (Qiagen). 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 inhibition on EV secretion, BMSCs were treated with 10 µM GW4869 (nSMase2 inhibitor, Sigma) for 48h. Results: MM-BMSCs andMGUS-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 from derived 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 andmgUS-BMSCs. It was confirmed that overexpression of miR-10a inhibited cell proliferation and induced apoptosis of MM-BMSCs, while the cell proliferation and apoptosis ofmgUS-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 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 expression have never been investigated in myeloma.

Aim: 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 mRNA and protein of PBK were detected by real-time RT-PCR and western blotting, respectively. The promoter activity 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 chosen using the online CRISPR design tool (www.eb dumpsters.de). Knockout of PBK was confirmed by quantitative real-time RT-PCR. Colony formation assay. The KMS-11 cells were subcutaneously injected to mice and were homogeneously CD73+, CD90+, CD105+, CD34-, and CD45-. Surprisingly, in the PBK-deficient KMS-11 cells, all of which carry PBKA/A, and OPM2 cells, which carry PBKG/G, augmented cell proliferation in the PBK-deficient OPM2 cells, which carry PBKA/A originally. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing PBKG/G compared with those cells expressing PBKA/A.

Results: In vitro anti-MM activity of therapeutic BIX01294 treatment was tested using the murine 5TGM1 model. Difference in overall survival between groups was determined in the UAMS-TT2 cohort of newly diagnosed MM patients (n=345), susceptibility and proliferation were examined by the MITT and colony formation assay. The KMS-11 cells were subcutaneously injected to mice and tumor volumes were observed every 3 to 4 days. Of note, PBK inhibition by CRISPR-mediated knockout enhanced cell proliferation in ANBL-6, 8226, and OPM2 cells, all of which carry PBKA/A. Surprisingly, in the

KMS-11 cells carrying PBKG/G, PBK inhibition by CRISPR-mediated knockout suppressed cell growth in vitro and in xenograft mice (Fig. 2). Moreover, exogenous expression of PBKG/G augmented cell proliferation in the PBK-deficient OPM2 cells, which carry PBKA/A originally. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing PBKG/G compared with those cells expressing PBKA/A.

Figure 1.

Summary/Conclusions: Our findings indicate that expression of PBKG/G was associated with myeloma cell proliferation, while PBKA/A was likely linked to tumor suppression. Increased phosphorylation of Thr 9 on PBK might contribute to proliferation in cells with PBKG/G. These results provide a novel insight into the mechanisms underlying myeloma cell growth and PBK is a promising target for the treatment of multiple myeloma.
E1212
CYTOTOXIC LYMPHOCYTES IN NEWLY DIAGNOSED MYELOMA HAVE REVERSIBLE FUNCTIONAL AND PHENOTYPIC ABNORMALITIES THAT MAY OFFER THERAPEUTIC OPPORTUNITIES
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Background: A bi-directional interaction exists between malignant cells and those of the immune microenvironment. This dynamic relationship results in gradual loss of clonal control associated with loss of cytotoxic lymphocyte (CTL) response. Mechanisms of immune escape are varied and include the induction of immune checkpoint molecules, notably the PD-1/PDL1 axis. Multiple myeloma is a disease characterised by a pre malignant phase which can evolve into periods of asymptomatic and symptomatic disease. One possible mechanism for disease progression is progressive loss of immunological control. The malignant plasma cell has multiple potentially immune modifying effects including the expression of PDL1 and induction of a pro-tumour micro-environment. The role of CTLs is less well understood.

Aims: To undertake deep immune profiling of the CTL landscape in myeloma in order to establish whether features of immune dysregulation are present and to identify potential therapeutic opportunities.

Methods: Cryopreserved bone marrow from 18 patients with newly diagnosed and untreated myeloma and 9 controls were assessed using a 36 parameter mass cytometry panel. The panel was designed to assess 9 immune checkpoint regulators, 5 cytokines, and markers of proliferation and degranulation across multiple lymphocyte subsets. Samples were stimulated with CD3 and CD28 to assess immune function. Dimensional clustering/algorithmic analysis was used alongside traditional data analysis techniques to identify functional subpopulations characterised by expression of multiple markers.

Results: The cytokine profile in newly diagnosed myeloma is shifted towards a pro-tumour microenvironment with particularly marked elevation of TGFb throughout resting CTLs (36.4% v. 66.2%, p=<0.0001). IFNγ production is reduced in the resting myeloma effector population (0.33% v. 0.18%, p=0.0099). Stimulation restores the cytokine profile to match that of controls. Myeloma CTLs retain the capacity to proliferate and produce the constituents for cytotoxic granule formation, however elevated PD1 expression alongside other markers of exhaustion cut NK cells away from the putatively exhausted phenotype is occurring. Strongly PD1 expressing populations in myeloma are larger (26% v.43%, p=0.05) and have increased rates of co-expression of CTLA4 (32%, 64%, p=0.0015), PD1L (26% v. 47%, p=0.0198) and TIM3 (34% v. 56%, p=0.0241). Populations of CTLs from myeloma up-regulate expression of the TCR co-stimulatory molecule CD40L (74% total CD8), NGK2D (45% total CD8) and OX40 (33% total CD8) following stimulation.

Summary/Conclusions: Clear differences can be identified in the functional and phenotypic features of CTLs in myeloma compared to those of controls. The partial nature of these defects and the fact that reversibility can be demonstrated suggests that these cells have not yet reached the stage of irreversible exhaustion. Taken together this data suggests that targeting immune checkpoint regulators at an early disease stage, in order to optimise immunological function and reverse partial defects, is a viable therapeutic strategy to explore. PD1, PD1L, CTLA4 and TIM are all potential immune checkpoint targets. In addition, our specific IgG antibodies BIX01294 and the UNC138 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 of myeloma cells is associated with a strong increase in the formation of LC3B puncta and an increase in LC3II and beclin-1 protein levels. In addition, we found that BIX01294 sensitizes MM cells to the proteasome inhibitor bortezomib and the Bcl-2 inhibitor ABT199. Lastly, therapeutic treatment of 5TGM1 inoculated mice with BIX01294 resulted in a clear improvement in survival, as evidenced by a clear decrease in tumor burden and a significant increase in the overall survival of BIX01294 treated mice compared to vehicle treated mice.

Summary/Conclusions: Altogether, our results demonstrate for the first time the importance of the histone methyltransferases G9a/GLP in MM pathogenesis. Further functional targeting of G9a/GLP induces MM cell apoptosis, enhances MM sensitivity to ABT- 199 and bortezomib and significantly delays tumor progression in the murine 5TGM1 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 signaling 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 tumour-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, KMS5, OPM2, Delta47, KMS11) were treated with 48 h CP (20 nM). Subsequently, 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, immunofluorescence 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 microscopy. 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 isolates from patients with IC50 values in the range of 2.5±11.2 µM. CP also suppressed the growth of MM xenografts in mice. Mechanistically, CP was found to induce intrinsic apoptosis in MM cells via increasing mitochondrial and cytosolic ROS production. Interestingly, CP-induced apoptosis occurs regardless of the cells’ p53 status, suggesting that CP has additional mechanisms of action. In addition, we found that CP acted synergistic with the protease inhibitor carfilzomib (CFZ) in MM cells, providing a framework for further studies of CP alone and in combination with CFZ to improve the prognosis for MM patients.

Summary/Conclusions: Our findings indicate that CP could be an attractive candidate for treatment of MM even in patients with p53 abnormalities; this study may identify an unmet clinical need, as such patients currently have a poor prognosis.

E1214
TUMOR MICROENVIRONMENT TRANSFORMATION FROMmugUS TO MYELOMA IS ASSOCIATED WITH PRO-TUMORAL ACTIVATION OF MESC- ENCHYMAL STROMAL CELLS (MSC)
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Background: A well-recognized feature of MM is the intimate relationship between plasma cells (PC) and bone marrow microenvironment, characterized by a modified extracellular matrix, enhanced angiogenesis and presence of cells with immune suppressive activity, including tumor-associated macrophages and myeloid-derived suppressor cells (MDSC). Recently, we have demonstrated that MM-MSC are able to convert normal immature myeloid cells in MDSC contributing to immune-escape mechanisms.

Aims: We hypothesize that MSC derived from Smoldering myeloma (SMM) and MM are in an activated status that promotes tumor growth and increase microenvironment transformation.

Methods: Human peripheral blood mononucleated cells (PBMC) isolated from SMM or MM-MSC. After 6 days, neutrophils (N) were isolated using anti-CD66b magnetic microbeads and were tested for their ability to induce angio-
Results: Only N educated by SMM- and MM-MSC (both from patients at diagnosis, relapsed and refractory) significantly up-regulated Arg1, NOSE2 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 Cell (HBMEC), we observed increased both tube length and number of branch points only in conditions where HBMEC were incubated with SMM- and MM-MSC. MM patients that displayed an angiogenic phenotype after achieving sCR were CT/PET negative.

vs

Results: [Results from the study are presented here.]

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 (1). 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 growth. Moreover, Notch signaling, with MM 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 microenvironment (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, cultured human endothelial cells (HPECs) were used for the stromal compartment. The GFP+HS5 cell line, Matrigel and wound healing assays were set up to investigate Notch role in modulating the angiogenic potential of MM cells co-cultured with HPECs and HPCAECs. HPECs in response to MM-derived soluble factors. To develop a TME-like system, a decellularized extracellular matrix (dECM) was used as a physiological scaffold for organoid generation. dECM was produced by treating murine fibroblast NIH3T3 with ascorbic acid and was loaded with cells for organoids generation. We evaluated apoptosis of MM cells in single culture and co-culture with BMSCs or HPCAECs by flow cytometry.

Results: [Results from the study are presented here.]
ARQ-197, a small-molecule inhibitor of c-MET, reduces tumour burden and prevents tumour-associated bone disease in a murine model of myeloma.

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 cells in monocultures were used as controls. Co-cultures were then applied to magnetic cell separation to isolate MM cells for downstream 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 of BMMSCs (BMSCs) 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 BMSCs up- or down-regulated several miRNAs and mRNAs in MM cells. Survivin (BIRC5) was confirmed to be conserved in different MM cell lines and protein and mRNA levels increased. 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 BMSCs were pre-treated with BFA but highly significantly when they were separated from MM cells with a TW insert. The same trend was observed in in vivo analyses FACS analysis indicating that direct cell-to-cell adhesion was more effective in BMSC-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 expression correlated with the number of BMMSCs in MM cell monolayers compared to tumour spheroid cultures. Furthermore, miR-101-3p overexpression or silencing of survivin increased BTZ-induced apoptosis in MM cells in the absence or presence of BMSCs significantly overcoming stroma-mediated drug resistance. To test whether miR-101-3p could also regulate adhesion of MM cells to BMSCs, we showed that miR-101-3p significantly reduced adhesion of MM cells to HS.5 and primary MM BMSCs compared to scrambled control. This finding suggests that miRNA-101-3p regulates cell adhesion-mediated drug resistance (CAMDR) by modulation of BM-MSC adhesion.

Summary/Conclusions: Our results identify a mechanism whereby BMSCs 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-miRNA-101-3p axis in regulation of BMSC-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

CHARACTERIZING THE CONTRIBUTION OF BONE MARROW StromA-DERIVED IL-6 TO myELOMA GROWTH AND RESISTANCE

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Background: The bone marrow niche is a specialized microenvironment, which allows for the survival, growth and differentiation of hematopoietic stem and progenitor cells. This niche also provides the optimal growth conditions for hematopoietic malignancies, such as multiple myeloma (MM). A complex interplay between cytokines, adhesion molecules, cell receptors and their ligands provides the MM plasma cells with survival signals and contribute to therapy resistance.

Aims: To unravel the role of the bone marrow mesenchymal/stromal cells (BMMSCs) in MM 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, myeloma 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, myeloma, and MM patients, as well as BMMSCs impacted by MM in our humanized bone marrow-like model, allowed us to confirm established disease biomarkers (e.g. IL-
CD38 monoclonal antibody, Daratumumab (DARA), induces and mediates MM cell killing. However, the expression of CD38 and other functionally related ectoenzymes in the bone niche of MM patients and the potential effects of DARA on bone cells are still unknown.

**Aims:** The aim of this study was to define the expression profile of CD38, CD31, CD39, CD73 and CD203a on MM and bone niche cells. Then, the effect of DARA on bone cells was evaluated by in vitro osteoclastogenesis.

**Methods:** In order to evaluate the expression profile of the ectoenzymes, we firstly performed immunohistochemical analysis on bone biopsies in a cohort of 37 patients with MM and 14 with monoclonal gammopathy of uncertain significance (MGUS). The same antigens were analyzed by flow cytometry on primary MM cells, mesenchymal stromal cells (MSC), osteoblasts (OB), monocytes and osteoclasts (OC). Then, we tested DARA effects, in the presence or absence of All-trans retinoic acid, compared with human IgG isotype control, on OC differentiation from either CD138+ cell fraction or purified MM bone marrow (BM) CD14+ cells. We also investigated the effect of microvesicles isolated from a MM cell line treated with DARA or the human IgG isotype control, on OC differentiation.

**Results:** MM cells showed a high expression of CD38 and were positive for CD31, CD39, CD73 and CD203a at variable levels. However, we did not find any significant difference in the expression of CD38 and related ectoenzymes between MM and MGUS patients. CD38 was expressed by monocytes and early OC progenitors but not by OB. MM cell expression of CD203a was higher for CD73 and CD203a. Indeed, CD38 was lost during OC differentiation. Consistently, we found that DARA reacts with CD38 expressed by monocytes and its binding inhibits early in vitro osteoclastogenesis from total mononuclear cells. All-trans retinoic acid treatment increased the inhibitory effect of DARA on OC formation.

**Summary/Conclusions:** Our data suggest that DARA inhibits osteoclastogenesis, targeting monocytes and early progenitors. These observations provide a rationale for the use of an anti-CD38 antibody-based approach as treatment for MM-induced osteoclastogenesis.

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THE PAN-PIM KINASE INHIBITOR, PIM447, POTENTLY SYNERGIZES WITH POMALIDOMIDE PLUS DEXAMETHASONE IN PRECLINICAL IN VITRO AND IN VIVO MODELS OF MULTIPLE MYELOMA

**E1222**

**Background:** Bone disease is the hallmark of multiple myeloma (MM). It is known that MM cells express CD38 and that a recently developed human anti-CD38 monoclonal antibody, Daratumumab (DARA), induces and mediates MM cell killing. However, the expression of CD38 and other functionally related ectoenzymes in the bone niche of MM patients and the potential effects of DARA on bone cells are still unknown.

**Aims:** The aim of this study was to define the expression profile of CD38, CD31, CD39, CD73 and CD203a on MM and bone niche cells. Then, the effect of DARA on bone cells was evaluated by in vitro osteoclastogenesis.

**Methods:** In order to evaluate the expression profile of the ectoenzymes, we firstly performed immunohistochemical analysis on bone biopsies in a cohort of 37 patients with MM and 14 with monoclonal gammopathy of uncertain significance (MGUS). The same antigens were analyzed by flow cytometry on primary MM cells, mesenchymal stromal cells (MSC), osteoblasts (OB), monocytes and osteoclasts (OC). Then, we tested DARA effects, in the presence or absence of All-trans retinoic acid, compared with human IgG isotype control, on OC differentiation from either CD138+ cell fraction or purified MM bone marrow (BM) CD14+ cells. We also investigated the effect of microvesicles isolated from a MM cell line treated with DARA or the human IgG isotype control, on OC differentiation.

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**Summary/Conclusions:** Our data suggest that DARA inhibits osteoclastogenesis, targeting monocytes and early progenitors. These observations provide a rationale for the use of an anti-CD38 antibody-based approach as treatment for MM-induced osteoclastogenesis.

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TRIM33 IS A POTENTIAL TUMOR SUPPRESSOR IN MULTIPLE MYELOMA

**E1224**

**Background:** TRIM33 is a potential tumor suppressor in multiple myeloma (MM). TRIM33 has previously been identified as a tumor suppressor in chronic myelomonocytic leukemia and hepatocellular carcinoma.

**Aims:** The aim of this study was to examine TRIM33 expression and to investigate its role as a potential tumor suppressor in MM.

**Methods:** Western blotting and qPCR were used to analyse TRIM33 expression at basal level and following knockdown in four MM cell lines representing a range of MM translocations; JNJ3 t(14;16), U266 t(11;14), KMS-18 t(4;14), OPM-2 t(4;14). TRIM33 knockdown was performed using shRNA plasmids. Cell Titer-Glo® was used to determine cell viability following knockdown of TRIM33 expression. Gene expression analysis was performed using a custom microarray containing 384 human genes associated with TRIM33 expression in non t(4;14) MM. Low TRIM33 expression has also been associated with poorer overall survival in patients with a t(4;14) chromosomal translocation.

**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 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 caused a significant increase in genes associated with TRIM33 expression in non t(4;14) cell lines, particularly (p=0.004) and hyperdiploid cluster (p=0.03). Low TRIM33 expression has also been associated with poorer overall survival (GSE2658; p=0.0034).

**Summary/Conclusions:** Our data suggest that DARA inhibits osteoclastogenesis, targeting monocytes and early progenitors. These observations provide a rationale for the use of an anti-CD38 antibody-based approach as treatment for MM-induced osteoclastogenesis.

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EXPRESSION OF CD38 AND ECTOENZYMES OF THE ADENOSINERGIC PATHWAYS IN MM EYE-BONE NERVE: A RATIONAL BASIS FOR THE USE OF DARATUMUMAB TO TARGET OSTEOCLAST FORMATION IN MULTIPLE MYELOMA

**E1223**

**Background:** Bone disease is the hallmark of multiple myeloma (MM). It is known that MM cells express CD38 and that a recently developed human anti-CD38 monoclonal antibody, Daratumumab (DARA), induces and mediates MM cell killing. However, the expression of CD38 and other functionally related ectoenzymes in the bone niche of MM patients and the potential effects of DARA on bone cells are still unknown.

**Aims:** The aim of this study was to define the expression profile of CD38, CD31, CD39, CD73 and CD203a on MM and bone niche cells. Then, the effect of DARA on bone cells was evaluated by in vitro osteoclastogenesis.

**Methods:** In order to evaluate the expression profile of the ectoenzymes, we firstly performed immunohistochemical analysis on bone biopsies in a cohort of 37 patients with MM and 14 with monoclonal gammopathy of uncertain significance (MGUS). The same antigens were analyzed by flow cytometry on primary MM cells, mesenchymal stromal cells (MSC), osteoblasts (OB), monocytes and osteoclasts (OC). Then, we tested DARA effects, in the presence or absence of All-trans retinoic acid, compared with human IgG isotype control, on OC differentiation from either CD138+ cell fraction or purified MM bone marrow (BM) CD14+ cells. We also investigated the effect of microvesicles isolated from a MM cell line treated with DARA or the human IgG isotype control, on OC differentiation.

**Results:** MM cells showed a high expression of CD38 and were positive for CD31, CD39, CD73 and CD203a at variable levels. However, we did not find any significant difference in the expression of CD38 and related ectoenzymes between MM and MGUS patients. CD38 was expressed by monocytes and early OC progenitors but not by OB. MM cell expression of CD203a was higher for CD73 and CD203a. Indeed, CD38 was lost during OC differentiation. Consistently, we found that DARA reacts with CD38 expressed by monocytes and its binding inhibits early in vitro osteoclastogenesis from total mononuclear cells. All-trans retinoic acid treatment increased the inhibitory effect of DARA on OC formation.

**Summary/Conclusions:** Our data suggest that DARA inhibits osteoclastogenesis, targeting monocytes and early progenitors. These observations provide a rationale for the use of an anti-CD38 antibody-based approach as treatment for MM-induced osteoclastogenesis.
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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Aims: To determine whether altered lncRNAs 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 (BMCs) of healthy donors as controls. We also performed ssRNA-seq of populations from B cell differentiation (Naïve, Germline Center, Memory and PC). To study the heterogeneity of lncRNAs expression we performed sample level enrichment analysis (SLEA), in which each individual lncRNA was compared to BMCs. To determine the epigenetic regulation of lncRNAs we used whole-genome bisulfite sequencing and CHIP-seq, shRNA-mediated knockdown using 2 different shRNAs and MTSS1 overexpression in 3 different cell lines (MM.1S, MM.1R and KMS-11)

Results: We identified 40.552 novel lncRNAs in MM samples that were present in at least 3 of the 38 patients. Principal component analysis demonstrated that TPCs and BMCPCs cluster separately, suggesting that, in spite of being the same tissue type, their transcriptomes are very different. We observed that the expression of lncRNAs 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 IncRNAs that appeared as deregulated when all MM were compared to BMCs, demonstrating the relevance of studying the heterogeneity in this disease. To determine the functional role of heterogeneously altered IncRNAs in the biology of MM cells we focused on the study of LINC-SMIL0 (Specific Myeloma inhibitor Long non-coding RNA), a lncRNA that it is overexpressed in ~40% of MM patients and not in the different stages of B-cell differentiation. DNA methylation analysis demonstrated that CpGs located upstream of LINC-SMIL0 showed a significant hypomethylation immGUS, that was even more pronounced in MM samples. We also have observed a gain of active chromatin modifications in the promoter region of LINC-SMIL0 in MM patient samples. These data suggest that epigenetic alterations, namely the gain of active histone modifications, may be the cause of LINC-SMIL0 overexpression in MM. Knockdown of LINC-SMIL0 in 3 different cell lines (MM.1S, MM.1R and KMS-11) resulted in reduced proliferation and induction of apoptosis, indicating this IncRNA 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, overexpression of LINC-SMIL0 is required for the survival of MM cells and could represent 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 is found to be overexpressed in many cancers, including some hematological malignancies (Keane et al. 2012). Furthermore, EphA3 has been found overexpressed not only in neoplastic cells, but also in the microenvironment of different human cancers, where its targeting inhibits tumor growth by disrupting supportive stroma and vasculature (Vail et al. 2014).

Aims: Due to the absence of relevant information about the role of EphA3 in multiple myeloma (MM), we aimed to evaluate the expression of this molecule in primary 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 shEphA3 versus shControl cells. Furthermore, the effects of EphA3mAb were analysed in a MM xenograft model by measuring tumor size and by assessing angiogenesis, proliferation and apoptosis rate on tumor biopsies using immunohistochemistry (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).

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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 77 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 (Cytocell). 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, 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 pts of FISH-analysis was performed also in disease progression.

Results: Chromosomal aberrations were revealed in 133 of 134 (99%) pts. T(1q14;1q23) was detected in 42.5% (57/134), hyperdiploidy in 57.5% (77/134), hypodiploidy in 2.1% (3/134) pts. In 11.2% (15/134) a concurrent t1q14h1q23 and a trisomy were found. The IgH translocations t(11;14), t(4;14), t(14;16), t(14;20), t(6;14), t(14;20) were observed at a frequency of 16.4%, 12.7%, 3.2%, 0.7% respectively, chromosomal partner is not found in 6.7%. Del(13q) was detected in 40.3% (54/134), del(17p) in 12.7% (17/134), tMYC/8q24 in 17.2% (23/134). Amp1q21 was detected in 39.6% (53/134). We identified 3 copies of 1q21 in 60% (80/134) and >3 copies 1q21 in 21 in 39.6% pts. Cases with Amp1q21 had a high incidence of del(13q) (OR=2.71 (1.32-5.55); p=0.006 and t(4;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).

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.

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 immunoturbidimetric sFLC were estimated 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 gammopathy of undetermined significance (MGUS), smoldering myeloma (SMM), and MM at diagnosis (MMD), throughout treatment (MMT) and at relapse (MRR). Quantification of circulating lymphocyte subsets showed reduced, absolute, numbers of B cells (56/102), T cells (19/102), TCI 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=0.094). No clearcut correlations were found between reductions in uninvolved and 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 NK cells, in keeping with reduced levels of circulating B cells, followed by T cells, particularly TH cells which have a crucial role in B cell Ig production. Relative B cell levels in BM were significantly correlated 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 DPPIV enzymatic 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 (OCG) 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.
Methods: Human BM-MNCs derived from normal human subjects or MM patients were cultured with M-CSF plus sRANKL with or without 250 nM CH-223191 for 48 h for OC formation for TRAP staining and functional assay. To assess the mechanisms of action of CD26Ab on OCs, RANK signaling proteins were examined by immunoblotting.

Results: CD26 is expressed on normal human OCs and is intensely expressed on activated OCs in MM, M cancer, and BM samples from multiple myeloma (MM) patients at first diagnosis. The cells were also stained with CD26 antibody to distinguish tumor CD26+ BM cells. After 72 hours, cells were stained with CD8 antibody to distinguish tumor CD26+ BM cells. CD26 expression was accompanied by increased phosphorylation of MKK3/6 and p38MAPK, which is crucial for human OC formation with its downstream activation of microphthalmia-associated transcription factor (mTrkA) and plays an important role in OC functions. CD26 blockade and down-regulation of RANK-L 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 CD26Ab, which blocks RANK activation, the OC precursors (MKK3/6) were involved in the phosphorlation pathway was specifically, rapidly inactivated and subsequently, its downstream miF/2a 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-mTrkA was not phosphorylated at all in mature OCs after RANKL stimulation, regardless of the absence or presence of CD26Ab. These results suggest that CD26Ab blocked RANKL induced p38MAPK phosphorylation in OC precursor cells, but not in OCs. The activation of other MAPKs including ERK and SAPK/JNK, or NFκB was rapidly induced in response to RANKL both in OC precursor cells and mature OCs, regardless of the absence or presence of CD26Ab. CD26Ab did not directly affect mature OC functions. Next, although CD26Ab 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 11 MM cell lines with 11 MM cell lines were performed. We examined the expression of CD26 in CD26 Ab. 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 examined the expression of CD26 in OCs and MM cells. The expression of CD26 was found in OCs in the BM of MM patients, which was consistent with the results of immunohistochemistry. We initially screened 25 CD138+ MM patients and 6 human myeloma cell lines (HMCs) for PERK mRNA expression. Our data showed that PERK mRNA is highly expressed in almost all patients (5-10 fold higher than the mean PERK expression of HMCs).

Results: We tested the effect of GS2606414 on the proliferation of MM cells. CD26Ab was treated with different doses of GS2606414 at two time points (24 and 48 hours). Treatment of cells with 3-30 µM GS2606414 resulted in a dose-dependent inhibition of cell proliferation in all HMCs ranging for 20-95% reduction of proliferative activity, thus, indicating the dependency of these cells on CD26 signaling. Treatment of M-CSF and sRANKL induced human OCs for 24 hours resulted 25% and 15% increase in apoptotic cells by Annexin-V staining. These results suggest that CD26 signaling is associated with increased the apoptosis of T-cells with high AhR, as assessed by Annexin-V staining (percent Annexin-V positive and 7-AAD negative cells in 0 and 25 µM kynurenine was 11.8% and 36%, respectively). We found that CH-223191 could block the inhibitory effects of kynurenine on T-cells. We found that the percentage of T-cells with high AhR in culture with IDO negative and positive Raji cells was 46.7% and 61.4%, respectively, while the percentage of T-cells with low AhR was 41.6% and 39.4%. These data showed that only T-cells with high AhR were inhibited at IDO-positive tumor microenvironment. Next, we could generated the activated T-cells from 10 of 12 BM samples from MM patients. We found that there was a positive correlation between the expression of AhR and the proportion of plasma cells in BM (r=0.76, P=0.04) and clinical stage of MM. The mean expression of AhR in T-cells of stage 2 and stage 3 were 7.3% (3.3-11.6) and 19.5% (11.3-26.6), respectively.

Summary/Conclusions: Kynurenine produced by IDO, induce inhibitory signal in T-cells through the AhR. Anti-CD4 and anti-CTLA-4 therapies, which block directly the inhibitory signal in T-cells, have been getting some clinical benefits against such as melanoma and Hodgkin lymphoma. Therefore, the AhR in T cells might be a target for IDO-positive hematological malignancies.
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 THERAPEUTIC TARGET

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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 work showed that actin(0.02, p<0.001) B cell-secreted and PC-specific Blmp-1, immunoglobulin (Ig) production and ATP availability (Pengo, 2013) and that MM is addicted to the autophagy receptor SQSTM1/p62 (Milan, 2015). Among putative pro-tumoral players are immunosuppressive BM-derived high-density neutrophils (HDNs), but their role in MM is unknown.

Aims: We hypothesized that in MM HDNs sustain PC fitness sustaining p62 through environmental arginine deprivation.

Methods: We integrated diverse unbiased and hypothesis-driven approaches: (1) gene expression profiles (GEP) of patient-derived circulating HDNs (60 MM, 30mGUS, 30 healthy controls), (2) metabolic profiling by UPLC/GC-MS of ad hoc collected BM and peripheral plasma (16 MM, 17 smoldering MM, 30mGUS, 29 controls), and (3) functional and expression analysis in vitro studies on human MM cell lines. We validated our observations in primary MM cells using bioinformatic analysis of transcript expression levels detected by RNA-sequencing available from the open-access, public clinical and molecular database, the CoMMpass Researcher Gateway (https://research.themrmf.org, v I48, n=649).

Results: In vitro, selective and progressive arginine deprivation (range 1000-0 µM) in four MM cell lines (MS 1S, U266, OPM2 and RPMI8282) activated the GCN2/CHOP axis, resulting in increased p62 and Blmp-1 expression, increased ATP availability and immunoglobulin production. Conversely, stable lentiviral p62 silencing significantly reduced Blmp-1 and ATP, and led to complete extinction of MM cell lines within 10 days of culture. Bioinformatic analysis of MMRF-Encompass trial data showed a positive correlation between p62 and Blmp-1 expression and OS (HzR: 0.38, 95% CI: 0.23-0.6, p<0.001). Both p62 and Blmp-1 expression 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 b40ml/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: 0.23-0.6, 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 eGFR are cheap and available for most of MM patients, therefore the CKD-EPI/ISS prognostic model will be widely applied. Nevertheless, the establishment of CKD-EPI/ISS model requires further validation.

E1233
ESTIMATED GLOMERULAR FILTRATION RATE (eGFR) CALCULATED BY CKD-EPI EQUATION COMBINED WITH THE INTERNATIONAL STAGING SYSTEM PROVIDES A POWERFUL PROGNOSTIC MODEL FOR EARLY MORTALITY IN MYELOMA PATIENTS

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Background: During the last decades, the introduction of autologous transplantation and novel agents has improved early mortality rates (EM, henceforth defined as death within year after diagnosis) in Multiple Myeloma (MM). However, the incidence of EM remains high. Data relating to prognostic factors for EM in MM are limited.

Aims: The aim of this study was to explore for possible prognostic factors for EM, which could be a useful tool for planning treatment strategy in MM.

Methods: We have studied the medical records of 479 patients with MM (MF: 258/221, median age: 68 years, range 29-88, IgG: 269, IgA: 123, light chain: 72, non-secretory: 15), diagnosed and treated in our Department between January 2001 and January 2016; 86 patients (18%) had EM. Comparisons of patients’ characteristics between the EM group and the rest of the patients were 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; hemoglobin, platelets and albumin were lower whereas W2 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 (<40ml/min/1.73m2) was higher in the EM group compared with the rest of the patients (60% 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 del(17p were present in 48% of patients in the EM group vs 21% of the rest of patients (p<0.001). The presence of EM included infections 54%, 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.

Based on our data, 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.

E1234
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 sorted and cocultured in 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 cytotoxicity with europium-TDA and cul- turing on DRCs in MM by side population (SP) detection.

Results: SP cells from both cell lines and samples from different stages of MM showed overexpression of stemness markers. Patient NKAES 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 did not affect the viability of the SP cells without significant differences. NKAES cells were also able to destroy CTCs from different MM cell lines. Cyto- toxicity studies revealed that NKAES cells did not destroy mononuclear cells from healthy bone marrow, even at maximum ratios of 32:1 the mean cytotoxicity was 1.85% (range 0 - 4.47%). Experiments on CD34+ hematopoietic progenitor cell cultures also showed that NKAES cells do not destroy CD34+ clones from healthy bone marrow, confirming the safety of NKAES.

Summary/Conclusions: NK cells have molecular characteristics of the tumor stem cell compartment in MM. Likewise, NKAES cells from MM patients could
destroy drug resistant MM cells and clonogenic tumor cells with high efficiency, preserving CD38+ hematopoietic cells, and thus constitute an effective and safe therapy against MM.

E1235

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 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 first exon 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 cell context-specific manner.

Methods: RepeatMasker software was used to reveal the presence of integrated TEs in the genomic segment comprising TNFSF11. RNA-Seq data, generated from the PRJEB4337 and PRJNA182351 BioProjects, were analyzed via the UCSC Genome Browser Database. Data on TNFSF11 proximal promoter methylation status in 63 cell lines were downloaded from the HAIB Methyl40k ENCODE track.

Results: RNA-Seq data from 51 normal human tissues show that sRANKL mRNA is expressed predominantly in normal human bone marrow and white blood cells. Methylation status of sRANKL mRNA in 5 lymphoblastoid cell lines (LCLs) signifies that the proximal 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, PA5X, 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 progression may be implicated 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 because this type of transcriptional boost could be the key mechanism driving 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 promulgating the anti-osteoclastogenic properties of IMiDs.

E1237

ADENOSINE AS A CHECKPOINT AND KEY PLAYER 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 inflammatory and immune responses. Multiple myeloma (MM), a plasma cell malignancy that develops within the bone marrow (BM) niche, overexpresses CD38, a molecule that catalyzes the initial disassembly of NAD (to cADPR and ADPR), which is followed by formation of ATP, GTP, and Ado. Ado production was likely due to the nucleotide-metabolizing enzyme, CD38, which 9 (41%) had a non-IgG peak, compared to 10/26 (38%) evolution in other high risk factors (a non-IgG monoclonal peak or a peak at >15g/L).

Methods: Evaluation of the expression of adenosinergic ectoenzymes was assessed by immunohistochemical and flow cytometric analysis on cell lines, and primary myeloma cells and BM biopsies from patients with MM or with monotypic immunoglobulin and bone marrow infiltration by plasmocytes, with no associated symptoms of Multiple Myeloma (MM). The risk of development of symptomatic MM justifies identification of the factors associated with an increased risk of evolution. Flow cytometric quantification of the ratio of bone marrow pathological/total plasmocytes (PP/PT) has been reported to be predictive in this context (Pérez-Persi et al. Blood 2007). The PP/PT ratios ≥95% were considered high risk. Disease evolution was indicated by a necessity to treat.

Results: The 210 PP/PT ratios were on average 77% (10-100). Amongst the 154mgUS patients, 24 had a ratio >95%, of which 8 (33%) evolved, compared to 9/130 (7%) with a ratio below 95%. Only 2 of these 8mgUS demonstrated other high risk factors (a non-IgG monoclonal peak or a peak at >15g/L). Amongst SMm patients, 22/30 (73%) patients with a high ratio evolved, of which 9 (41%) had a non-IgG peak, compared to 10/26 (38%) evolution in SMm with low PP/PT ratios. The risk of evolution to active MM was significantly higher in patients with a PP/PT >95%, both in thigmUS (p=0.0001) and overall (p=0.0004). There was a discordance between PP/PT ratio and disease evolution in 11% (17/154)mgUS patients and 23% (48/210) of the overall group but no other FC markers associated with an increased risk of evolution could be identified.

Summary/Conclusions: We confirm the clinical value of a simple, rapid, two-tubes FC quantification of the proportion of pathological plasma cells in the evaluation of the risk and kinetics of disease evolution mgUS and SMm. It's use allows identification of patients which require more frequent follow-up.

E1238

THE RATIO OF PATHOLOGICAL PLASMACYTES, ASSESSED BY 8-COLOR FLOW CYTOMETRY, PREDICTS RISK OF DISEASE PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND SMouldERING MULTIPLE MYELOMA
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Background: Deregulation of the expression of CD38+ monoclonal immunoglobulin and bone marrow infiltration by plasmocytes, with no associated symptoms of Multiple Myeloma (MM). The risk of development of symptomatic MM justifies identification of the factors associated with an increased risk of evolution. Flow cytometric quantification of the ratio of bone marrow pathological/total plasmocytes (PP/PT) has been reported to be predictive in this context (Pérez-Persi et al. Blood 2007). The PP/PT ratios ≥95% were considered high risk. Disease evolution was indicated by a necessity to treat.

Methods: All patients undergoing bone marrow evaluation following identification of a monoclonal peak (at diagnosis or during follow-up) during a 7.5year period with a diagnosis ofmgUS (n=154) or SMm (n=56) and at least 6 months follow-up were analysed by 8-color FC (including 11 antibodies) from fresh whole bone marrow. Patients with PP/PT ratios ≥95% were considered high risk. Disease evolution was indicated by a necessity to treat.

Results: The 210 PP/PT ratios were on average 77% (10-100). Amongst the 154mgUS patients, 24 had a ratio >95%, of which 8 (33%) evolved, compared to 9/130 (7%) with a ratio below 95%. Only 2 of these 8mgUS demonstrated other high risk factors (a non-IgG monoclonal peak or a peak at >15g/L). Amongst SMm patients, 22/30 (73%) patients with a high ratio evolved, of which 9 (41%) had a non-IgG peak, compared to 10/26 (38%) evolution in SMm with low PP/PT ratios. The risk of evolution to active MM was significantly higher in patients with a PP/PT >95%, both in thigmUS (p=0.0001) and overall (p=0.0004). There was a discordance between PP/PT ratio and disease evolution in 11% (17/154)mgUS patients and 23% (48/210) of the overall group but no other FC markers associated with an increased risk of evolution could be identified.

Summary/Conclusions: We confirm the clinical value of a simple, rapid, two-tubes FC quantification of the proportion of pathological plasma cells in the evaluation of the risk and kinetics of disease evolution mgUS and SMm. It's use allows identification of patients which require more frequent follow-up.

22nd Congress of the European Hematology Association
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 marrowstromal (BMS) cell line (HS-5 cells) and primary samples from newly diagnosed MM patients. Apoptosis analysis was done up to 48h 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.

Summary/Conclusions: Schedule-dependent synergistic cytotoxicity was demonstrated for the combination of CAR and POM and a maximal apoptosis consistently observed in POM pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models using low dosage of both drugs. Whilst the clinical efficacy of CAR and POM combinations has been demonstrated, the synergistic cytotoxicity may be further exploited by using optimized schedule. Utilizing such a schedule with IMiDs pre-treatment may improve the depth and duration of response of MM patients both as upfront therapy and in the relapsed/refractory setting.
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 DPa, DRd, and Dv respectively. Eighteen (14%) patients received ‘other’ DCT. Median time to first response (≥ PR) was 3.1 months (95% CI 1.9-4.6). Overall response rate was 46%, [complete response + partial response] + very good partial remission + complete response not reached (NR). Median progression-free survival was 5.5 months (95% CI 4.2-6.8). Median duration of response was 6.1 months (95% CI 4.1-7.8) from starting DCT. Median overall survival was 67 (43-93) months. Among patients who had received 2 or more DCTs, median overall survival was 51.3 (5-156) months. 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 (OR: 0.38, 95%CI: 0.20-0.73, p<0.04). There was a trend toward longer overall survival in the Metformin group, but it was not statistically significant (170 vs 160 months, p<0.10). In a multivariate analysis of adverse events associated with the use of metformin, mSMART risk was associated with a better progression-free survival (OR: 0.38, 95%CI: 0.20-0.73, p<0.001).

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 clinical 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 grouped based on bony and soft tissue disorders. 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 plasmacytoma.

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 bicalonal myeloma), 7 had ISS stage I disease with a median paraprotein concentration 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 differences in the detection of vertebral L5 (using diffusion weighted MRI 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 colocoler cancer.

Summary/Conclusions: We have shown no difference in PET-CT and WBMRI in detecting a myeloma defining focal bone lesion, or providing prognostic estimators 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 significant residual disease among those who attain complete response (CR). The role of MRD in AM 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) and a three light chain ratio (for IgA). For flow cytometry, bone marrow samples were processed following the EuroFlow Bulk Lysis Standard Operating Protocol and stained with the EuroFlowIMF MM MRD panel. At least 5x10⁶ events were measured using a FACS Canto II (USA) instrument. Data were analyzed using the Infinicyt software (Spain). Patients were identified as having residual disease if a discrepant 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 (41%) had cardiac involvement at diagnosis. More than 2 lines of treatment 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 versus negative patients, (p=0.08). 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. However, improvement of cardiac function compared to the time of CR was observed in all 5 evaluable MRD- patients and in none of the 2 MRD+ patients (P=0.047). Compared to the time of CR, renal response was obtained in 7 MRD- subjects (84%) and in 4 (50%) MRD+ (P=0.153). Overall, further improvement of cardiac or renal function after CR was significantly associated with a lower ADC at ≤0.60mm²/s (P=0.012).

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 in patients in CR.
patients at induction. A number of factors have been shown to correlate with overall survival (OS) and progression free survival (PFS) including depth of remission prior to ASCT, initial ISS stage and high risk cytogenetics. Emerging evidence has demonstrated that early relapse following ASCT is associated with reduced OS, and is not correlated with depth of pre-transplant response.

Aims: To characterise myeloma patients who relapsed within 12 months of ASCT, through baseline characteristics and transplant engraftment, and assess the impact of this early relapse on OS and PFS.

Methods: We performed a multicentre retrospective analysis of patients who underwent ASCT at 3 centres between 01/2009 – 02/2016 (London) and 06/2006 – 03/2013 (Cardiff). Baseline characteristics were reviewed and ASCT engraftment was assessed; by time to neutrophils 50 x 10^9/L and platelets >20 x 10^9/L. Post-transplant PFS & OS was calculated by time (months) from diagnosis to progression or death.

Results: 443 myeloma patients were identified, median age was 57 (r 31-73), 56% were male. 41% of patients were ISS stage 1, 34% stage 2, 25% stage 3. Cytogenetic data was available for 139 patients. 1st-line therapy prior to transplantation was immunomodulatory drug (IMiD) based (THAL/LEN) for 318/443 patients & 72/443 were proteasome inhibitor (PI) based (BORT/CARF). In addition, 11 patients received combination PI and IMiD. Median time from start of therapy to ASCT was 10 months (r 3-109m), 67 patients progressed within 12m of ASCT (early progression). No statistical difference was found between <12m or >12m relapse for; age, gender, 1st line therapy, ISS stage, Hb, LDH, Ca or cytogenetics, confirming that this group is difficult to predict at baseline. Median OS from time of diagnosis was 103 months (95% CI 101 -137), median OS from start of ASCT was not reached, however 5-year OS was 68%. Patients with progressive disease within 12 months of ASCT, has significantly reduced median OS: 12 months (95% CI 21-39) compared to non-progressive patients (median OS:1039m 95% CI 89-117).p=0.0005. Median OS from ASCT was reduced in early progression median OS:18m (95% CI 14-22m) vs progression >12 months median OS:89 months (95% CI 79-98m) p=0.0005. 1st line therapy did not influence likelihood of PFS<12months, with no statistical difference between patients who received PIs, IMiDs or both 1st line (p=0.484). A significant difference was observed in median time to platelet engraftment between the 2 centres. Increased time for platelets to reach >20 x10^9/L was associated with reduced OS from ASCT for each centre HR 1.14 & 1.20 (p=0.046 & 0.03) for Cardiff or London centres respectively (Cox’s Method).

Summary/Conclusions: Early relapse following ASCT is a significant predictor of inferior OS in myeloma and difficult to predict from standard baseline characteristics. From our analysis; 1st line treatment prior to ASCT did not influence OS or PFS. There was an association between slow platelet engraftment following ASCT and PFS and OS. Possible explanations include: residual occult disease, toxicity of chemotherapy or patient stromal factors which facilitate disease resistance and impair normal haematopoiesis. All of these factors have been shown to drive relapse. RCCs are required to standardise bone marrow response assessment post ASCT, quantify remission status (using laboratory and imaging techniques) and definitively predict early relapse. Additionally, these studies will investigate further biological or genetic mechanisms driving early relapse to help identify novel therapeutic approaches in these extremely poor prognosis group.

E1246

PATIENT-REPORTED OUTCOMES (PROS) WITH IBRUTINIB: SUBSTUDY OF INNOVATETM FOR WALDENSTROM MACROGLOBULINEMIA (WM)


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Background: Anemia and fatigue are frequent indications for WM treatment. To date, patient-reported outcomes (PROs) have not been used to quantify benefits of ibritinib (ibr), a first-in-class, once-daily inhibitor of BTK, is indicated in the EU for the treatment of WM after ≥1 prior therapy or >12m relapse for: age, gender, 1st line therapy, ISS stage, Hb, LDH, Ca or cytogenetics, confirming that this group is difficult to predict at baseline. Median OS from time of diagnosis was 103 months (95% CI 101 -137), median OS from start of ASCT was not reached, however 5-year OS was 68%. Patients with progressive disease within 12 months of ASCT, has significantly reduced median OS: 12 months (95% CI 21-39) compared to non-progressive patients (median OS:1039m 95% CI 89-117).p=0.0005. Median OS from ASCT was reduced in early progression median OS:18m (95% CI 14-22m) vs progression >12 months median OS:89 months (95% CI 79-98m) p=0.0005. 1st line therapy did not influence likelihood of PFS<12months, with no statistical difference between patients who received PIs, IMiDs or both 1st line (p=0.484). A significant difference was observed in median time to platelet engraftment between the 2 centres. Increased time for platelets to reach >20 x10^9/L was associated with reduced OS from ASCT for each centre HR 1.14 & 1.20 (p=0.046 & 0.03) for Cardiff or London centres respectively (Cox’s Method).

Summary/Conclusions: Early relapse following ASCT is a significant predictor of inferior OS in myeloma and difficult to predict from standard baseline characteristics. From our analysis; 1st line treatment prior to ASCT did not influence OS or PFS. There was an association between slow platelet engraftment following ASCT and PFS and OS. Possible explanations include: residual occult disease, toxicity of chemotherapy or patient stromal factors which facilitate disease resistance and impair normal haematopoiesis. All of these factors have been shown to drive relapse. RCCs are required to standardise bone marrow response assessment post ASCT, quantify remission status (using laboratory and imaging techniques) and definitively predict early relapse. Additionally, these studies will investigate further biological or genetic mechanisms driving early relapse to help identify novel therapeutic approaches in these extremely poor prognosis group.

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-505, IST-CAR-501).

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) (CCyd), followed by carfilzomib maintenance until progression or intol-
Pomalidomide (POM) + Low-Dose Dexamethasone (LoDEX) Following Second-Line LEN-BASED Treatment in Pts with RRMM: Results of the IST-CAR-561 Trial

**Methods**

- **Patient Population:** 148 pts with a median age of 72 years were analyzed. At enrollment, 34% of pts 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 pts; any grade hypertension was reported in 17% of pts, dyspnea in 9%, and heart failure, arrhythmia and venous thromboembolism (VTE) in 6% of pts, each. Grade 3-5 CV-AEs occurred in 15% of pts. 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).

- **Aim:** 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 on day 1, 8, 15 and 21; 45% of pts, hypertension and dyspnea were the most common. Pts ≥75 years had a higher risk of any grade (58% vs 36%, p=0.002) 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.

**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 carfilzomib. 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 48.0% 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). Common grade 3/4 adverse events (AEs) included anemia (25.5%), neutropenia (11.8%), and infections (19.6%; including pneumonia [8.8%]. 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).

- **Response Type:**
  - ≥ PR (n = 15)
  - Stable Disease (n = 21)

<table>
<thead>
<tr>
<th>POM Treatment Duration, months</th>
<th>≥ PR</th>
<th>Stable Disease</th>
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<tbody>
<tr>
<td>6 months</td>
<td>3</td>
<td>12</td>
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<tr>
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<td>24 months</td>
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**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 in use in later treatment lines. In addition, achieving disease control of ≥ MR led to similar PFS rates as reaching ≥ PR.

**Table 1:**

- **POM Treatment Duration, months**
  - ≥ PR
  - Stable Disease

**E1249“REAL WORLD” DATA ON THE EFFICACY AND SAFETY OF IZAXOMIB IN COMBINATION WITH CARFILZOMIB AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A STUDY OF THE GREEK MYELOMA STUDY GROUP**

**Background:** The overall combination of ixazomib, lenalidomide and dexamethasone (IRd) has been recently approved as a novel standard of care for relapsed/refractory multiple myeloma (RRMM). The aim of this study was to evaluate the efficacy and safety of IRd in the “Real World” (RW) practice, where data are very limited.

**Methods:** This was a retrospective, non-interventional study, which recorded IRd treatment data from patients with RRMM who participated in the name-patient program of ixazomib in Greece. The primary endpoint was the evaluation of overall response rate (ORR) and response rate among treated pts. Secondary endpoints included: treatment duration; time to response; duration of response; percentage of patients who experienced adverse events (AEs), needed dose modification or treatment discontinuation; evaluation of PFS and TTP.

**Results:** Forty-one patients were included in the present study. Of those, 35 (85%) (median age 67-79 years; range 63-79 years) had received at least 3 cycles of IRd on the date of data analysis and thus they were included in the present report. The median line of previous therapies was one (range: 1-5): 71.4% (25/35) patients had received one prior treatment, while 20.0% (7/35),...
and 4 with other therapies. Baseline characteristics were balanced across 3557 patients (98.0%) had discontinued treatment. Of the 73 patients (2.0%) received bortezomib (BORT), 137 (3.8%) received thalidomide (THAL), and 155 (4.3%) lenalidomide (LEN).

**Results:**
Patients provided informed consent. Up to 36 mos after treatment discontinuation (Protocol amendment 2011). All laxis was administered per local standard practice. AEs were graded per the ground cohort (all other treatments, including novel agents). Thromboprophylaxis is provided and patients gain access to new therapeutic approaches.

**Summary/Conclusions:** LEN was generally well tolerated and the safety results were similar to published data. As expected, the occurrence of neutropenia, TCP, and VTEs were higher in patients in the LEN cohort, whereas neuropathy was more frequently reported in patients in the BORT cohort. VTEs were low in all cohorts. The occurrence of SPMs was generally low and comparable between cohorts.

**Background:** Clinical outcome of multiple myeloma (MM) patients is heterogeneous and depends on various prognostic factors and available treatments. Although tremendous progress has been made in MM, so far, there is no national or international evidence-based guideline giving recommendations for clinical practice in the treatment of MM patients. In Germany, 14 Comprehensive Cancer Centers (CCC) are funded as Centers of Excellence by the German Cancer Aid (DKH). All these Centers of Excellence are required to develop and provide in-house clinical pathways for standards in cancer care. These pathways include concise diagnostic and therapeutic instructions, reflecting available evidence-based recommendations. In addition, ongoing studies (in particular phase I/II) are part of clinical pathways, so that a rapid transfer of innovation is provided and patients gain access to new therapeutic approaches. The Centers of Excellence Network working group SOP has the goal to harmonize these hospital specific in-house clinical pathways differently in format, content and level of evidence. In addition, ongoing studies (in particular phase I/II) are part of clinical pathways, so that a rapid transfer of innovation is provided and patients gain access to new therapeutic approaches. The Centers of Excellence Network working group SOP has the goal to harmonize these hospital specific in-house clinical pathways differently in format, content and level of evidence. In addition, ongoing studies (in particular phase I/II) are part of clinical pathways, so that a rapid transfer of innovation is provided and patients gain access to new therapeutic approaches. The Centers of Excellence Network working group SOP has the goal to harmonize these hospital specific in-house clinical pathways differently in format, content and level of evidence.
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 ccw.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.

Figure 1.

Summary/Conclusions: The first clinical MM pathway developed and released by all 14 German CCCs/Centers of Excellence translates scientific evidence and expert knowledge into precise suggestions for MM patients in clinical practice. These harmonized processes might increase the quality of cancer care throughout Germany. To be up-to-date and to reflect latest research findings this clinical pathway will be updated every 18 months.

E1252

WT1 HETEROCLITIC EPITOPE 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 for a mutation of a WT1 peptide fragment (RMFPNPAYL)-HLA-A*0201 complex on the engagement interface between malignant plasma cells and T-cells in HLA-A*02+ 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 galinpepimut-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 (fb) lenalidomide maintenance starting 3 months (mos) post-SCT. 13/16 pts presented with high-risk (HR) cytogenetics [t(4;14), t(14;16), del17p, 1q21/25 gain and/or del13q]. We report initial results from MM pts immunized with the WT1 heteroclitic peptide mixture galinpepimut-S (GPS) after autoSCT.

Results:

- 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 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 delineate the influence of SOs and other factors (evaluated at 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 that identify predictors for PFS and OS. Furthermore, we aimed to identify clinical/therapeutic features predicting the occurrence of PD before ASCT.

Methods: Non-trial MM patients who had undergone a single or tandem ASCT at the University Hospital of Heidelberg in the years 1992-2014 were analyzed regarding their response before 1st ASCT (d=0) 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).

Summary/Conclusions: Our analysis provided valuable insights into the response before 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 with 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. Response was evaluated according to EBM7-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.

E1254

SEVERE INFECTIONS IMPACTS OVERALL SURVIVAL IN ACTIVE MULTIPLE MYELOMA PATIENTS

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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, PD is a significant factor for occurrence of PD at time of 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.
**REAL-WORLD SETTING IN THE UNITED STATES**

**DIFFERENT TREATMENT MODALITIES OF MULTIPLE MYELOMA IN THE EVALUATION OF CARDIOVASCULAR EVENTS ASSOCIATED WITH E1255**

Background: Multiple myeloma (MM) represents the second most common hematological malignancy characterized by the proliferation of monoclonal plasma cells (PC) in the bone marrow. The natural history of active MM patients may be complicated in significant fraction by the occurrence of infections that can be related both to the development of therapy induced neutropenia (mainly due to high dose chemotherapy used in the setting of autologous stem cell transplantation or in salvage regimens) or to MM induced secondary immunodeficiency.

Aims: The aim of this study was to analyse the frequency, the type and the major risks factors of severe infections in our cohort of patients affected by MM and to understand the impact of these events on MM patient overall survival (OS).

Methods: A cohort of 341 patients affected by MM (104 with smouldering MM and 237 with symptomatic MM) followed from 1996 to 2016 was retrospectively studied for the presence of severe infections (si, defined by the need of hospitalization) during the natural history of the disease. Infections were classified as “not neutropenia related” or “neutropenia related” according to the Absolute Neutrophil Count > or <1,000/ml respectively. International Staging System (ISS) and Durie-Salmon (DS) were used for MM patients staging.

Results: In our cohort of patients, si were significantly associated to active MM (28.69% of symptomatic patients vs 3.85% of asymptomatic patients; p=0.0001, c²=25,318). Among the 138 infective events occurred in 91 active MM patients, 38 (26%) were neutropenia related while remnant 100 not neutropenia related (72%). Furthermore, almost 44% of these events (61/138) developed during induction therapy, with 12 out of 61 (20%) being present at time of the diagnosis. Considering that majority of si was not neutropenia related and that these infective events involved most of active MM patients who developed si (68/91, 75%), our aim was to identify MM patient characteristics associated to the development of not neutropenia related si. Our results prove evidence that major features presented at the time of the diagnosis significantly associated to si were DS stage III (p=0.0004, c²=12,14), ISS stage III (p=0.0001, c²=21,11), age >70 years (p=0.0195, c²=5,455), bone marrow plasma cells >60% (p=0.034, c²=4,50), acute renal failure (p=0.0003, c²=13,010) or MM presenting with at least three of CRAB criteria (p=0.0123, c²=6,26). For what concern the impact of si on the natural history of the disease, patients who experienced infective event presented a reduced OS towards other patients (p<0.0001). Among infected patients no significant differences were reported referring to the number of infections (>1 or =1, p=0.11), while patients who developed exclusively neutropenia related infective events showed better OS towards patients who experienced not neutropenia related infections (p=0.0011).

Figure 1.

Summary/Conclusions: Severe infections represent an underestimated comorbidity in MM, characterizing all phases of the disease and not only refractory/relapsed patients receiving multiple lines of therapy. Considering that severe infections impact OS mostly in the setting of not neutropenia related infections, immunoglobulin replacement therapy and/or antibiotic prophylaxis may possibly have a protective role in high risk old patients characterized by ISS and DS stage III, bone marrow PC >60% and aggressive disease at the time of diagnosis.

**E1255**

**EVALUATION OF CARDIOVASCULAR EVENTS ASSOCIATED WITH DIFFERENT TREATMENT MODALITIES OF MULTIPLE MYELOMA IN THE REAL-WORLD SETTING IN THE UNITED STATES**

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Background: Multiple myeloma (MM) is a disease of the elderly. The prevalence of cardiovascular (CV) comorbidities in the MM population is high. Past research suggests that MM is associated with a range of cardiac risks, and emerging evidence shows that both proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) can have important CV sequelae. The improved efficacy of PI plus IMiD combination therapy (PI+IMiD) has resulted in its widespread adoption, which suggests that CV events may become a prominent concern in patients receiving PI+IMiD as contemporary treatment for MM.

Aims: To assess the risk of developing CV events in patients receiving anti-MM treatment and to less a specific treatment modality was associated with higher risk of a CV event.

Methods: Patients with ≥1 inpatient claim or ≥2 outpatient claims with a primary diagnosis code for MM who were treated with PI and/or IMiD drugs between Jul 2012 and Sep 2014 were identified in a large US claims database. The first claim for a PI or IMiD drug in this period was defined as the index date, which was preceded by 180-d continuous eligibility with no anti-MM treatment (base-line). Patients were divided into three cohorts based on the anti-MM treatment received: PI, IMiD, PI+IMiD. CV events of interest included cardiac arrhythmia, cardiac failure, venous thromboembolism (VTE), myocardial infarction, ischemic heart disease, angina, stroke and coronary atherosclerosis, and were measured during anti-MM treatment. Kaplan–Meier methods were used to estimate the occurrence rate of a CV event, and multivariate Cox regression models were developed to identify prognostic factors of each CV event among patients treated with anti-MM therapies.

Results: 4288 patients met the eligibility criteria for inclusion in the study (57% male, median age 66 y, 41% with Charlson Comorbidity Index ≥2, mean duration of treatment 192 d; Table). 42% (n=1779) were treated with PIs, 38% (n=1624) with IMiDs and 20% (n=865) with PI+IMiDs. Patients receiving PI+IMiD were significantly younger and generally had lower prevalence of CV comorbidities than those receiving PI or IMiD (Table). Compared with patients on PI, the risk of developing VTE was 46% greater in patients on PI+IMiD (HR: 1.46; 95% CI: 1.09, 1.96). Compared with those on IMiD, the risk of developing cardiac failure and cardiac arrhythmia was 33% and 18% greater in patients on PI+IMiD (HR: 1.33; 95% CI: 1.03, 1.72; HR: 1.18; 95% CI: 1.00, 1.40). After 6 months of treatment, the rates of VTE were 8%, 10%, and 11% for patients on a PI, those on an IMiD and those on PI+IMiD, respectively. The corresponding rates for cardiac failure were 18%, 11% and 11% for PI, IMiD and PI+IMiD cohorts, and 21%, 16% and 22% for cardiac arrhythmia.

Table 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 used lenalidomide or combinations with thalidomide (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 for the treatment of newly diagnosed MM.
Methods: We collected the clinical data of 169 patients qualified to the Study Group as follows: bortezomib: 1.3 mg/m² (days 1, 4, 8, 11), thalidomide: 100 – 200 mg a day (days 1, 2, 4, 5, 8, 9, 11, 12) or 40 mg 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.

Results: In the cohort of 169 patients, median age was 59 years (range 36-70). ISS stage 1 was found in 30.8% of patients, ISS 2 and 3 in 20.7% and 45.5%, 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 polyneuropathy as the most common reason (75%). Polyneuropathy was also the most common grade 3/4 adverse event, observed in 20 patients (11.8%) and neutropenia 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 plasmapheresis/ cyclophosphamide (42% of patients). In 69.3% of patients one apheresis allowed to obtain the number of stem cells sufficient for transplantation. Median yield of CD34+ cells was 11 x 10^6/kg (max 55.7x10^6/kg) that was sufficient for two transplantations in the majority of patients. HDT/autoSCT was performed so far in 89 patients with MEL 200 protocol as conditioning regimen in 77.6% of patients. Median number of transplants 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 performed in 81 patients, sCR rate increased from 5.6% to 12.7% and CR from 27.1% to 36.7%.

Summary/Conclusions: VTD regimen allowed to achieve ≥ PR in 94% of patients including ≥ VGPR in 84.5% of patients as compared to 73.5% ≥ PR including 36% of ≥ nCR achieved in patients treated with CTD in our previous study (Dmoszynska et al. Leuk Res 2010). In 96% of patients subsequently undergoing stem cell mobilization sufficient number of CD34+ cells was obtained during first procedure. HDT/autoSCT further increased response rate after VTD induction (≥ CR up to 43.5%, ≥ VGPR up to 83.5%).
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 2006 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 (63.2%) were transplant ineligible while 26 (36.8%) were eligible. 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 isotype 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 (VGPR) or better was achieved in 53.6% of patients. Three-year OS rate was 50.4%. IP was observed in 27.5% of the patients at diagnosis. Patients with IP had a higher bone marrow plasma cells (BMPC) infiltration (29 vs 9; P<0.001). Also a trend towards a higher difference between involved and uninvolved free light chains was observed in the group of patients with IP (360.2 vs 221.7; P=0.08). IP was more frequent in those who received an ASCT (57.5% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (39.13% vs 34.78%; P=0.4).

Regarding its prognostic value, IP did not influence survival in the whole series. In the ASCT group, the presence of IP resulted in a significantly shorter PFS (median: 30.2 months vs not reached [NR]; P=0.019; Figure 1A) and OS (62.5 months vs NR; P=0.097). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs NR; P=0.047; Figure 1B), but not significantly different in OS. Multivariate analysis restricted to the population of patients with stage I and II Mayo12, incorporating ASCT, BMPC and IP, indicate that IP retained its independent prognostic factor for worse PFS (HR=12.06; 95% CI, 1.9-75.7; P=0.008).

Figure 1.

Summary/Conclusions: The presence of IP has a negative impact on survival, especially in the sub-group of patients in early stages of the disease. The presence of IP at diagnosis could be an additional powerful discriminatory prognostic indicator in the group of patients without advanced stage of the Mayo risk stratification system of 2012.
Background: Polyclonal antibodies against the conformational epitopes between the heavy and light chains (HLC) of immunoglobulin (lg) have been recently introduced as diagnostic tool in multiple myeloma (MM) and other monoclonal gammopathies. They separately identify the two different light chain types of each lg, allowing the quantification of the monoclonal component. HLC and HLC ratios may be particularly useful for monitoring the presence of monoclonal component in oligo-secretory MM or when it migrates in the β region, as frequently observed in IgA MM. The International Myeloma Working Group (IMWG) has published in 2016 new consensus criteria for assessing response and minimal residual disease (MRD) in MM, outlining the potential role of HLC assay in this setting and the need of its further investigation, particularly in patients achieving complete response (CR) and not support a role for HLC as putative biomarker of MRD.

Methods: Twenty-five consecutive patients were evaluated. Mean age was 63 years (range 43-92), fourteen patients were males. Ig isotype was IgG or IgA in 14 and 11 patients, respectively, with 20 patients showing kappa and 5 lambda light chains. According to International Staging System, seven patients had stage 1, ten stage 2 and eight stage 3. Fourteen patients not eligible to autologous stem cell transplantation (AuSCT) received a bortezomib-based treatment, mainly constituted by bortezomib, melphalan and prednisone combination (VMP), while eleven patients underwent AuSCT after induction therapy with bortezomib, thalidomide and dexamethasone (VTD). With a median follow-up of 52 months (range 21-92), overall survival (OS) of the entire cohort was 61 months (95% CI 43-82), fourteen patients were males. Ig isotype was IgG or IgA and k/λ ratios were then calculated.

Results: At CR time, we found seven (28%) samples still showing abnormal HLC ratio and fourteen samples (56%) with abnormal FLC ratio. Discrepancy between the two assays occurred in 11 patients. FLC assay normalization in CR was significantly associated with better PFS (43 months, 95% CI 14-45 vs 12 months, 95% CI 5-25, p=0,03). In contrast, normalization of HLC ratio had no impact on PFS (26 months, 95% CI 10-38, vs 20 months, 95% CI 10-30, p=0,51), even selecting IgA MM. Notably, in 9 patients, the negative effect of abnormal FLC ratio at CR on PFS was not mitigated by concomitant normalization of HLC ratio (19 months, 95% CI 4-35, p=0,02). Neither FLC, nor HLC affected OS. There were no differences between patients who received AuSCT and those who did not.

Summary/Conclusions: To the best of our knowledge, this is the first study to analyze HLC ratios exclusively in MM patients in CR. While our preliminary data confirm the prognostic usefulness of FLC in this setting, currently they do not support a role for HLC as putative biomarker of MRD.

E1262
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 AE occurred, 3/26 pts (11%) treated with daratumumab in clinical practice had their therapy interrupted due to complications.

E1263
REAL-WORLD TREATMENT PATTERNS AND PATIENTS CHARACTERISTICS IN MULTIPLE MYELOMA ACROSS EUROPE
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Background: Emerging resistance to modern antimyeloma agents such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) remains the main clinical problem in managing multiple myeloma (MM). Daratumumab, a first-in-class anti-CD38 monoclonal antibody, has been recently approved in Europe as a monotherapy for patients (pts) with relapsed and refractory multiple myeloma (RRMM), whose prior therapy included a PI and an IMiD and whose disease had progressed during this time; based on the SIRIUS clinical trial outcome (Loniol et al. Lancet 2016). Nevertheless to optimize daratumumab use in clinical practice, more data on its “real life” activity and safety are still required.

Aims: This observational study of the Polish Myeloma Group (PMG) was aimed to prospectively evaluate the efficacy and toxicity of daratumumab monotherapy in RRMM. Last, daratumumab compassionate use named DaraCUP was used to treat patients in Poland.

Methods: Patients were eligible for DaraCUP if they met all the following criteria: a) confirmed diagnosis of RRMM, b) relapse after a minimum of 3 prior lines of therapy including PI and IMiD or were double refractory to both PI and IMiD (a and b) or were double refractory to both PI, IMiD and dexamethasone (a, b and c); 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 (56.3%) 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 PR) 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 median OS were not 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 inflammation (n=1), dyspnoea (n=1). The most common haematological toxicities were grade one anaemia (n=30.7%), neutropenia (n=23.1%), thrombocytopenia (n=18.5%), and neutropenic fever (n=9.2%). The most common laboratory abnormalities were grade one hypercalcemia (n=40%), hyperuricemia (n=24.1%), and grade one hypomagnesemia (n=14%). Among the 22 pts (73%) who had received at least 2 cycles of daratumumab, 21 pts (95%) 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.

Table 1.
Background: Multiple myeloma (MM) is the second most common haematological malignancy after non-Hodgkin lymphomas, accounting for 13% of blood malignancies and 1% of all cancers. 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=711); Austria, Switzerland, Belgium, Norway, Sweden, Switzerland and Finland (Central and Northern Region, CNR, n=776); Croatia, Estonia, Hungary, Latvia, Lithuania, Poland, Serbia, Slovakia (Eastern Region, ER, n=689). Analyses 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 transplantation (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 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 (2.6 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 bortezomib-based regimens were most frequent (38%). The median duration of second line therapy was shorter in SR and CNR than in ER (Table). Moreover, the 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.

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.

Table 1.

<table>
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<th>Multivariate Analysis</th>
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<td>Age years</td>
<td>1.09 (1.05-1.3)</td>
<td>&lt;0.001</td>
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<td>Charlson Comorbidity index</td>
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<tr>
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<tr>
<td>ISS</td>
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<tr>
<td>Presence of Renal Failure</td>
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<tr>
<td>Tipo de QT Alaplanter</td>
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<tr>
<td>IMIDs</td>
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<td>0.11</td>
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<tr>
<td>Bortezomib</td>
<td>1.23 (0.45-3.37)</td>
<td>0.68</td>
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</table>

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 uncontrolled secretion of monoclonal 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 nephrotic syndrome. However, in the presence 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 bortezomib.

Methods: A total of 133 patients (61 from ile-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 2:Univariate and Multivariate Cox Regression Analysis

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<th>P value</th>
<th>HR (95% CI)</th>
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<td>Presence of Renal Failure</td>
<td>1.06 (0.9-2.6)</td>
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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<0.05). Age (HR: 0.281, 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 TIME POINT FDG UPTAKE IN FOCAL LESIONS CORRELATE 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, Radeberg, Germany). Focal malignant lesions were localized in pre-treatment scans; maximum standard uptake value (SUVmax) and mean SUV (SUVmean) and partial volume corrected SUVmean (pvcSU-Vmean) were obtained for each lesion. Lesional response to chemotherapy was classified as complete or partial in the post-treatment scan. A complete response was defined as a complete resolution of the lesion in the post-treatment scan. Lesions with partial response were present in the post-treatment scan. All statistical analyses were done in SPSS 24 using repeated measurements-ANOVA.

Results: Three-five focal lesions were evaluated in each patient. In the pre-treatment PET studies, the increase in SUVmean from 1 to 3 hours was significantly higher for lesions with partial response compared to those with complete response (27.7% vs 11.4%; P=0.050). Additionally, the increase in pvcSU-Vmean was more significant than the increase in SUVmean (±42.23% vs +12.0%; P=0.003). The increase in SUVmax of delayed scans was not significant (P=0.082).

Summary/Conclusions: These preliminary data show that a more significant increase of FDG uptake in delayed scans of DTP PET before treatment correlates with better response to chemotherapy in MM. The increase in pvcSU-Vmean is a better index than those of SUVmean and SUVmax for this purpose.

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 tumor survival, drug resistance and the development of bone disease. The Notch oncopathic pathway provides a key contribution to the ability of MM cells to shape the BM niche, affecting both BM cell biology and the interplay between MM cells and the BM stromal cells. Extracellular vesicles (EVs) have been reported as novel mediators in creating a supportive milieu for MM. Here we investigate the role of the activated Notch signaling in EV-mediated cross-talk.

Aim: The idea 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 milieu for MM cells, with a focus on the contribution of EVs to the crosstalk between MM cells and the BM stromal cells.

E1269

REAL-WORLD DATA ON THE TREATMENT OF RELAPSED/REFRACTORY MYELOMA WITH LENALIDOMIDE AND DEXAMETHASONE IN 2ND LINE (LEGEND STUDY): THE PROGNOSTIC SIGNIFICANCE OF BIOCHEMICAL VS. CLINICAL RELAPSE


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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 which is 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 parameters for 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 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 31-91). In 67% (95% CI: 61.1% >73.9%) of patients and at clinical relapse in 32.5% (95% CI: 26.1-38.9) of patients. The overall response rate (ORR) was 73.4%: 23.7% of patients achieved very good partial response (VGPR) and 17.8% complete response (CR). The number of patients that achieved at least VGPR did not differ between the 2 groups (p>0.05). The median time to best response was 6.7 months (range 0.6- 51.9). After a median follow-up of 52.8 months, 112 (54.1%) patients are alive and 95 (45.9%) patients are deceased; 131 patients (63.3%) have relapsed (biochemical relapse: 66.4%, clinical relapse: 33.6%). Median PFS and PFS rate at 12 months was 19.2 months (95% CI: 15.6-25.2) and 67.6% respectively. The median PFS was 24 months (95% CI: 18.0-34.8) for patients in group A vs 13.2 months (95% CI: 8.4-19.2) for patients in group B (HR: 0.63, p=0.006). When adjusted for important prognostic patients’ characteristics (ISS, age, β2 microglobulin, and LDH), biochemical relapse maintained its prognostic significance for PFS (p<0.05).

Summary/Conclusions: Our data confirm that LenDex combination as 2nd line treatment leads to high overall response rates and prolonged PFS. Additionally, we have shown for the first time in routine clinical practice that MM patients who receive 2nd line therapy with LenDex at biochemical relapse have a significantly longer median PFS compared to patients treated at clinical relapse, underlying the importance of potentially starting treatment before evident clinical manifestations at the first relapse.
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 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 or eastern blotting.

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. SDF1α), 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. This is particularly relevant due to the crucial role played by bone disease in MM progression.

Summary/Conclusions: These new insights in the pathophysiology of the rearranged 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.

E1269
THE USE OF CARFILZOMIB AND BORTEZOMIB IN ROUTINE CLINICAL PRACTICE: RESULTS FROM PREAMBLE, AN ONGOING, OBSERVATIONAL COHORT STUDY IN MULTIPLE MYELOMA
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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 have documented the prevalence of hematological relapse and duration 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.

Aims: In this subsequent PREAMBLE analysis, treatment patterns in patients (pts) with RRRM receiving bortezomib (bort) and carfilzomib (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, ≥1 prior treatment and initiated treatment (index therapy) with an IMiD, PI or IMiD+PI within 90 days before to 30 days after study enrollment. Treatment patterns, DoT and time to next treatment (TTNT; for pts who switched or died) were assessed. Informed consent was obtained for all pts.

Results: At data cut-off (Sept 1, 2016), data were available for 924 pts, of which 326 (35%) pts had bort-based index therapy and 86 pts (9%) received carf-based index therapy (63/72 [88%] were enrolled in North America). The most common carf-based therapies were carf alone (n=40, 47%), which 326 (35%) pts had bort-based index therapy and 86 pts (9%) received carf-based index therapy (63/72 [88%] were enrolled in North America). The most common carf-based combination was bort + dexamethasone (dex; n=99, 30%). The most common carf-based therapies were carf alone (n=40, 47%), followed by carf+dex (n=21, 24%). The most widely used bort dose per 21 days for any bort-based therapy was 1.4 mg/m2 (28/55; 51%). Switch from carf-based index therapy occurred after a median (Q1, Q3) DoT of 3.4 (1.9, 9.5) mo (n=34; most pts switched to pomalidomide (pom)-based regimens (23/34; 68%). Switch rates increased from 17% at 3 mo to 57% at 33 mo, respectively; most pts switched to lenalidomide (38/99; 39%). In pts with carf-based index therapy, most commonly fatigue (12%) and anemia (9%); 70% (69/99) of pts receiving bort+dex had AEs, most commonly thrombocytopenia and diarrhea (each 14%).

Summary/Conclusions: Treatment duration observed for PIs in the real-world clinical practice setting was shorter than reported in clinical trials. As patient enrollment and follow-up continues for PREAMBLE, additional analyses will be conducted to evaluate the impact of these patterns on efficacy outcomes. Study funding: BMS.

E1270
ROLE OF SERUM FREE LIGHT CHAIN VS BENCE JONES MEASURE- MENT IN LIGHT CHAIN MULTIPLE MYELOMA (LCMM) AT DIAGNOSIS, DURING TREATMENT AND FOLLOW-UP FOR RESPONSE EVALUATION AND RELAPSE DETECTION
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Background: According to IMWG recommendations for response assessment in multiple myeloma (MM), serum free light chain (sFLC) measurement should be used to define a “stratified” complete response in symptomatic MM and, only in cases when Bence Jones protein (BJP) is deemed as not quantifiable (<200mg/24h), in light chain multiple myeloma (LCMM). However, data are available suggesting that sFLC could be a more sensitive tool than BJP for minimal residual disease assessment and an earlier indicator of progressive disease (PD). BJP measures shorter than sFLC, shorter than urinary electrophoresis and immunofixation, it is time-consuming for technicians and could be limited by poor patient compliance.

Aims: Aim of our study was to retrospectively compare sFLC and BJP results in LCMM patients (pts) at diagnosis, during treatment and follow-up.

Methods: Serum and urine samples were collected from pts affected with plasma cell dyscrasia referring to the Azienda Ospedaliero-Universitaria Careggi between 1st February 2012 and 31 December 2013. Serum and urine protein electrophoresis was performed using Capillaries II, serum and urine immunofixation using Hydrasys II (both from Sebia), sFLC were measured on Immage 800 nephelo-immunoanalyser using FreeL light chain reagents (The Binding Site).

Results: We analyzed samples from 387 pts having positive serum and/or positive urinary immunofixation and/or abnormal sFLC ratio. Among them, 43 symptomatic LCMM pts were identified having both sFLC and BJP measurement at baseline (at MM diagnosis or first relapse). Serum and urine lab tests results were evaluated at baseline, monthly during therapy and every 3 months during follow-up. Median duration of laboratory monitoring for the whole group was 42 months (range 3-120). Autologous stem cell transplantation was performed in 39% of pts previously treated with proteasome inhibitors (81%) and/or immunomodulating agents (40%) or chemotherapy (9%). sFLC or BJP were not available in 10% of 872 pair of samples from 43 pts. In 10% of cases (68/696 pair of samples) sFLC ratio was abnormal with involved FLC with no detectable BJP (FLC<f;FLC+BJP); the opposite (FLC>f;FLC+BJP) occurred in 1% of cases (8/696 pair of samples). Renal failure was found in 9% vs 13% of disreputable cases. At baseline, of the 43 LCMM pts, 13% had progressive disease only by sFLC due to BJP-200mg/24h and were therefore considered not evaluable for response assessment. Median time to BOR was 3 months by both sFLC and BJP (range FLC: 1-11 mesi; range BJP: 1-10 mesi). Among the remaining 37 pts evaluable for best overall response, 6/37 had complete response according to BJP but not to sFLC, interestingly 4 pts progressed after 2-8 months. Twenty-one pts progressed during follow-up: PD was detected only by sFLC in 4, only by BJP in 1. Both tests were able to detect PD in 16 pts: at the same time in 5, with sFLC-PD occurring earlier in 7 and BJP-PD occurring earlier in 4 pts.

Summary/Conclusions: Both sFLC and BJP results are useful in LCM MM pts for disease monitoring, however, sFLC assessment appears to be more sensitive in MRD and early relapse identification. These data suggest that BJP could be substituted by sFLC assessment in LCMM. In our series only 1 case showed BJP-PD according to IMWG occurring earlier than sFLC-PD but was considered not clinically significant. On the contrary 5 pts in BJP-6 had “measurable disease” within months without having reached FLC-GR. Limits of our study are a small number of pts, inhomogeneous duration of therapy and follow-up and retrospective analysis.

E1271
SUPPRESSION OF THE NON-MONOCLONAL PAIR AS NEW BIOMARKER FOR MONOCYTO MAJOR RESPONSIBLE MINOR RESPONSIBLE MM PATIENTS AT DIAGNOSIS AND AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION
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Background: The outcome for patients with Multiple Myeloma (MM) is highly 520 | haematologica | 2017; 102(s2)
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 abnormalities (CAs) at diagnosis as an OS predictor, as these are not routinely measured in practice; 2) adding 2L treatment as a predictor, as 2L treatment type is likely to affect OS; 3) changing the number of stratification groups.

Methods: The analyses used data for 1418 patients aged ≥18 years who were diagnosed with symptomatic MM between May 2007 and April 2016 and who had reached 2L. The Cox model was re-run for two sensitivity analyses: excluding CAs and adjusting for treatment received at 2L (adding bortezomib or lenalidomide vs other treatments as a predictor). The impact of different numbers of risk groups was assessed using KAPS.

Table 1.
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-life setting. It collects detailed data on patients. Diagnosis occurs at all institutions from 2012, nationwide. Analysis of this non-selected data will provide real-life information to plan strategies to improve MM management and perform 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.

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 at each institution. 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 to subtype was: IgG 50.4%, IgA 23.3%, Light chains 18.7%, non-secretor 2.2% and IgM <1%. Most patients had advanced disease: 79.6% Durie-Salmon stage III (176/221), 48.6% ISS (83/177). Anemia (hemoglobin ≤10 g/dl) was present in 48.6%, 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 45.8% (>70y vs 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. 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 hemolitic 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 patients 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 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
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 1.**

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>2013 MM Prevalence (%)</th>
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<tbody>
<tr>
<td>White</td>
<td>10.139</td>
</tr>
<tr>
<td>Asian</td>
<td>3.938</td>
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<td>Black</td>
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<tr>
<td>Unknown</td>
<td>2.740</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 INDUCED NEUROPATHY IN MULTIPLE MYELOMA AND ITS INFLUENCE ON PHYSICAL AND ROLE FUNCTIONING**

B, Sidi Bel Abbes

**Background:** Peripheral neuropathy (PN) is a major dose limiting and potentially disabling adverse event of commonly therapeutics drugs used in the management of multiple myeloma (MM), including the immunomodulatory drugs (IMiDs, Thalidomide and Lenalidomide), and the proteasome inhibitor (Bortezomib).

**Aims:** The aim of this study was 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-Neuotoxicity (Ntx) subscale for assessing treatment induced PN was evaluated. The 11-item of this questionnaire was administered to patients with MM treated with IMiD and/or Bortezomib. The subscale was evaluated in 32 participants for internal reliability, construct validity, criterion validity, and compared with other validated inventories (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 QLQ-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 NCi-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 IMiD (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<0.0001). R=0.0001 respectively.

**Summary/Conclusions:** The 11-item FACT/GOG-Ntx subscale reliably and validly assesses Bortezomib/IMiD 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**


**Background:** RRs of the immunoglobulin heavy chain (IGH) on chromosome 14 are identified by FISH in about 15-20% of patients (pts) with newly diagnosed multiple myeloma (MM). Historically there is little evidence that such rearrangements have a clinically significant impact on prognosis of these rearrangements: typically, t(4;14), t(14;16) and t(14;20) have high risk (HR), and t(11;14) have standard risk (SR). A recent study (Kaufman et al. Leukemia. 2016 30:633-9) suggests that t(11;14) may confer a worse prognosis.

**Aims:** To determine the prognostic significance of t(11;14) in a single-institution MM cohort.

**Methods:** 87 pts with t(11;14) by CD 138 selected FISH at diagnosis were identified, pts without symptomatic MM were excluded. Cox regression was used for statistical analysis. Progression free survival (PFS), and overall survival (OS) from diagnosis and post autologous stem cell transplant (ASCT) were analyzed by Kaplan-Meier.

**Results:** Median age at diagnosis was 62 years, 45 pts (52%) were male, and 24 pts (27%) had ISS 3. All pts received either a proteasome inhibitor or an immunomodulatory agent, and 62 (48%) received triplet treatment as induction. Sixty-nine (79%) pts had ASCT, and overall response rate (ORR, partial response or better) post ASCT was 73%. For pts with HR FISH (defined as t(14;16), p53 del, 1q21 gain or 1p del) compared to SR FISH, the ORR post ASCT was 70% vs 77% (p=0.67). OS from diagnosis was 93% at 3 years, 74% at 4 years and 51% at 5 years. Seven patients (6%) developed plasma cell leukemia, and there was no association between HR and SR FISH (p=0.66).

**Conclusion:** Despite the use of novel therapies the OS at 5 years of our pts with MM was not significantly improved compared to SEER data from 1992-2013 (51% vs 48.5%). Pts with t(11;14) who had ASCT had increased survival compared to those who did not. Our results suggest that t(11;14) may confer a worse prognosis. Further prospective studies evaluating the risk of t(11;14) are warranted.
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<.0005; Table). OS was also significantly improved with LEN-only vs no maintenance (HR=0.45 [95% CI: 0.29, 0.73]; P=.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. Dejoi 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 —mainly— urine (u) after treatment, given the usual difficulties to collect and adequately perform urine 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) 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 immunofixation was performed for the Ig, λ, k and λ chains (SAS-3 and SAS-4, Helena Biosciences Europe).

Results: From a total of 168 patients with measurable BJ disease (2013 Bence Jones 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 paraprotein in urine. [Figure 1A]. The concordance between the classification of the response by uPEP / immunofixation (IF) and by FLCs (Kappa Index=0.425 [95% CI: 0.001, 0.849]) was moderate. The normalization of the sFLC ratio (r) =<0.101 was achieved in 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].

Figure 1.

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 situation where many different treatment combinations are used, without a clear indication which patient will benefit most from which treatment. It is increasingly recognized that genetic heterogeneity between tumors influence 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 not (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 expected survival difference with the genetically most similar patients who received another treatment. Genetic similarity is defined separately for 10 581 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 as these 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 training set (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).

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 (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=11] >11 years). A significant percentage of patients had multigorgan involvement (54.7% [n=75] kidney; 29.9% [n=41] nerve; 14.6% [n=20] liver; 43.8% [n=60] heart; 43.8% [n=60]) 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 more than 75% of patients visited 3 or more different physicians before diag-

Table 1. Patient and Caregiver Survey Responses, %

Summary/Conclusions: This represents the first survey compiling both care-
giver 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
EFFICACY 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 expected regulating approvals.

Results: 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 expected regulating approvals.

Table 1. NMA Efficacy Results.

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 had investigated the efficacy of other treatment options considered to be comparators to DRd or Dvd. Data from trials that met the SLR’s inclusion criteria and the most recent data from POLLUX and CASTOR were extracted and then 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

haematologica | 2017; 102(s2) | 525
immunomodulatory agent (IMiD)–free regimens. Analysis using a fixed-effects model found that DRd compared with other IMiD-containing regimens in Network 1, and DVD compared with other IMiD-free regimens in Network 2 produced PFS and OS among patients 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 MMA suggests that the combinations of DRd and DVD may be active in RRMM 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 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 (Pt) 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 after 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 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, 717 (21.5%) received PI monotherapy, and 556 (19%) 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 (CyBod; n=124, 20.1%), lenalidomide + d (Rd; n=70, 11.3%), and bortezomib + d (Vd; n=57, 9.2%). In NSCT Pts (n=2307), the most common 1L regimens were RVd (n=642, 27.8%), Vd (n=510, 22.1%), and Rd (n=412, 17.9%). Among the RVd NSCT Pts with a documented 2L (n=189), the most common 2L regimens were CyBod (13.2%), carfilzomib monotherapy (7.4%), pomalidomide + d (6.9%), and Vd + d (6.9%). Documented death occurred in 785 (23.3%) Pts during the study period.

Summary/Conclusions: Over time, RVd has become the most common 1L regimen for SCT and NSCT Pts with ndMM. After a median follow up of 15.9 months, many patients remain in the initial LOT. With a longer follow up, we will be able to observe sequencing and patterns of treatment in later LOTs.

E1283
HLC PAIR SUPPRESSION AS A RISK FACTOR FOR BLOODSTREAM INFECTIONS AND EARLY DEATH IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS
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Background: Infection is a major cause of morbidity and mortality in patients with Multiple Myeloma (MM), responsible for 10-25% of deaths within 6 months of diagnosis. It is associated with suppression in primary antibody response (systemic immunoparesis (SI)), which may be aggravated following anti-myeloma therapy. Recently, the suppression of the non-monoclonal immunoglobulin pair (e.g. IgG-Lambda in IgG-Kappa MM), termed heavy+light chain (HLC) pair suppression, has been associated with poor prognosis in MM. However, the impact of HLC pair suppression as a risk factor for bloodstream infections has not been studied.

Aims: To evaluate HLC pair suppression as a risk factor for bloodstream infections and early death in MM patients.

Methods: The study retrospectively included 114 consecutive MM patients with diverse bloodstream infections, identified during investigation into unexplained fever and hospitalization in newly diagnosed MM patients. HLC pair suppression was defined as a positive blood culture related to a febrile episode. The population consisted of 66 male:48 female patients, with a median age of 68 (56-77) years. The monoclonal immunoglobulin isotype was: 49 IgG-K, 26 IgG-L, 21 IgG-A and 18 IgA-L. In order to explore the impact of HLC pair suppression on bloodstream infections and early mortality, only events within 6 months (180 days) from diagnosis were documented. HLC pair suppression was defined as suppression in the levels of the non-monoclonal pair by >50% below the lower limit of the reference range. HLC measurements were carried out using HCVYlite® immunomassays (The Binding Site) on a SPAPLUS analyzer. SI was defined as levels of the alternate immunoglobulin (e.g. IgA and/or IgM in an IgG patient) more than 50% below the lower reference range. Association between variables were analysed by Chi-square test and survival was estimated using Kaplan-Meier method.

Results: At diagnosis, HLC pair suppression was observed in 72 (63%) patients, and SI in 52 (45%). The incidence of bloodstream infections 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 vs those without suppression (34% 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 recognizing 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 1-2 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 men and 60 women, 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).


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 RESPONSE ASSESSMENT IN PATIENTS WITH MULTIPLE MYELOMA WITH VGPR OR BETTER
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Background: Urine and serum Immunofixation electrophoresis (ufIE 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)/oligosecreting 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, Kamagawa-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 multicolour 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 >200 were analysed. Normal FLC at VGPR, cCR and MRD-CR was 65.0%, 78.4% and 76.8% 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 in IIMM/OSMM was significantly high in 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%/94.8% and 100%/95.0%, respectively, while those were 90.6%/45.8% and 88%/32.4% in IIMM, respectively.

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 routine 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 SAVAGE THERAPY IN RELAPSED/REFRACTORY PATIENTS WITH MULTIPLE MYELOMA AFTER AUTOALLLOGENEIC 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 with the use of daratumumab in post-transplant setting.

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 autografts, 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 of salvage lines post-transplant and prior to first daratumumab infusion was 3 (1-4). The salvage regimens included bortezomib, lenalidomide, pomalidomide and daratumumab. Daratumumab infusions were started at a median of 21 months (0-30) after relapse/progress.

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 rush (CTC2, n=1), pressure on eyes (n=1). Two patients developed late onset infections (pneumonia and infection of urinal tract) followed by temporarily therapy interruption. We observed a decrease of Tregs (CD4+CD25highCD127low, n=15) number 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/progression 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 and 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.

E1287
PROGNOSTIC RELEVANCE OF VEGF AND VEGFR EXPRESSION IN CD138+CD19- AND CD138+CD19+ PLASMA CELLS FROM PATIENTS WITH MONOCLONAL GAMMOPATHIES
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Figure 1.
Results: 482 pts were eligible, 343 (71%) were W, 52 (10%) H, 50 (10%) B, 19 (4%) A, and 18 (3%) O. Median age was 65 years, 54% were male, and 28% ISS stage 3. There were no statistically significant differences in FISH aberrations between the M. Overall W had more aberrations in IGHR (4,14,14, t(11;14), t(14;20)), 1q21 gain compared to M. Most notably W had more IGHR (39% vs 28%, p=0.019) and t(11;14) (20% vs 12%; p=0.024). There were not statistically significant differences between W and M in the high risk FISH abnormalities.

| Table 1 |

<table>
<thead>
<tr>
<th>FISH</th>
<th>W</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomy 13</td>
<td>105 (60%)</td>
<td>46 (24%)</td>
</tr>
<tr>
<td>1q21 gain</td>
<td>22 (12%)</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>11q13 loss</td>
<td>30 (16%)</td>
<td>12 (6%)</td>
</tr>
<tr>
<td>13q14 gain</td>
<td>23 (12%)</td>
<td>10 (6%)</td>
</tr>
<tr>
<td>17p13 loss</td>
<td>19 (10%)</td>
<td>13 (7%)</td>
</tr>
<tr>
<td>t(4;14), t(11;14), t(14;20)</td>
<td>58 (31%)</td>
<td>17 (9%)</td>
</tr>
</tbody>
</table>

$p$-value compares W vs. M. * means statistical significant ($p$-value<0.05)

Summary/Conclusions: W had significant differences in FISH compared to M. W had more IGHR and t(11;14) than M, and there was no difference in high risk FISH aberrations between W and M. This study confirms the biological racial disparities that exist in minorities with MM. Further studies with more inclusion of minorities are needed to elucidate these disparities and its effects on risk stratification and outcomes.

E1289

**POMALIDOMIDE ALONE OR IN COMBINATION WITH LOW DOSE DEXAMETHASONE AS MAINTENANCE INDUCTION WITH POMALIDOMIDE AND LOW DEXAMETHASONE IN RELAPSING AND REFRACTORY MYELOMA (ALLG MM14)**

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**Background:** Whilst the addition of dexamethasone to upfront therapy with Immunomodulatory (IMiD®) agents is important to mediate rapid reduction in disease burden, preliminary findings suggest that the NK stimulatory effects of IMiD® compounds are best harnessed without the co-administration of dexamethasone, and may be especially effective in the setting of minimal disease burden (in the maintenance setting for example) when some inherent immune recovery has occurred. However this has yet to be confirmed in a prospective clinical trial.

**Aims:** To evaluate the effect of maintenance with POM alone (Arm 1) versus POM-LoDEX (Arm 2) on progression free survival (PFS), overall survival (OS), and kinetics of response (overall response rate (ORR)) in relapsed myeloma (MM) patients refractory to lenalidomide (R-LEN) demonstrating stable disease (SD) or better after first line therapy (bortezomb-based regimens for fit patients 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 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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**Background:** Racial disparities of FISH abnormalities in multiple myeloma (MM) have been well described in whites (W) but partially described in minorities (M) (Paulus et al, ASH 2016, 4432).

**Aims:** To explore the differential differences of FISH abnormalities using the largest cohort of minorities to date.

**Methods:** CD-138 selected FISH was done on 799 consecutive patients (pts). Pts without symptomatic MM, and biopsy >6 months after diagnosis were excluded. The abnormalities evaluated included standard and intermediate risk aberrations: t(4;14), t(14;14), t(11;14), t(4;14), t(14;20), del(13q), del(17p), 1q21. Chi-square was used for statistical analysis. Due to smaller numbers, all M (Hispanic (H), Black (B), Asian (A) and Other (O)) were included into the same group for statistical analysis.
Figure 1.
Summary/Conclusions: In patients with relapsed myeloma, after initial disease control/delubukation is achieved with Pom-LoDEX induction, maintenance with single-agent POM may be more effective for sustaining disease control than continuation of POM-LoDEX. Correlative studies are currently underway to further investigate the immunological mechanisms behind this observation.

E1290
POMALIDOMID IS MORE EFFECTIVE IN REAL CLINICAL PRACTICE THAN IN RANDOMIZED TRIAL – AN OBSERVATIONAL STUDY OF THE CZECH MYELOMA GROUP
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Background: The combination of pomalidomide and low-dose dexamethasone (Pom-Dex) is a perspective option for patients with end-stage relapsed/refractory multiple myeloma (RRMM). We analyzed efficacy and toxicity of Pom-Dex in all patients from the Czech Republic treated from June 2013 to December 2016.

Methods: Patients were eligible if they had been diagnosed with RRMM and had failed at least two previous treatments with bortezomib and lenalidomide. They were treated with start dose of Pom (4mg/day on days 1-21, orally) plus low-dose dexamethasone (40mg/day on days 1, 8, 15, and 22, orally) until disease progression or unacceptable toxicity. We analyzed TTP and OS together with toxicity. Also, univariate Cox proportional hazards model for OS was done for standard risk factors. One hundred and twenty-two patients with median age of 67 treated with Pom-Dex were evaluated. Median follow-up was 8.7 months. Median of previous treatment lines was 4.

Results: Median TTP of Pom-Dex treatment was 7.1 months (95% CI 5.3-8.6). Median OS was 19.0 months (95% CI 13.2-25.8). The most common grade 3-4 adverse events were neutropenia in 44%, anemia in 22% and thrombocytopenia in 24% of patients. Grade 4 infections were observed in 10% of patients. Patients with ECOG worse than 2, B2microglobulin higher than 5, ISS stage 3, low hemoglobin, low platelet count and presenting extramedullary mass had worse OS according to univariate Cox proportional hazards model.

Summary/Conclusions: Our analyses show that Pom-Dex treatment of Czech RRMM patients is effective, well tolerated and had better results than the registration study. Performance status and tumor burden seem to be main prognostic factors according to our model. Thus, our suggestion for clinical practice is to start pomalidomide treatment as soon as possible in case of MM relapse.

E1291
UNDERSTANDING THE REAL-WORLD CLINICAL CHARACTERISTICS OF MULTIPLE MYELOMA PATIENTS IN EUROPE
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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 Myeloma UK in Q1 2015. The Multiple Myeloma DSP is a real-world, cross-sectional survey that involves haematologists and haematology-oncologists who completed patient record forms for the next 8 multiple myeloma patients with whom they consulted. Study variables included patient demographics and background clinical information.

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/m². In terms of performance status, 79.8% of patients had an Eastern Cooperative Oncology Group (ECOG) status of 0 or 1, whereas 20.2% had an ECOG status of ≥2. While 12.9% of patients had smouldering myeloma, 47.5% of patients had advanced stage (stage III) disease. The most common symptoms experienced by patients were anaemia (31.0%), bone pain (32.4%), fatigue/weakness (28.4%), and kidney impairment or failure (12.6%). Furthermore, 34.6% of patients had bone complications at some point in time. Overall, 51.1% had diabetes.

Summary/Conclusions: Results from this analysis provide valuable insight into multiple 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% (6 (13.3%) 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 19.1 months (range: 11.0-24.9), 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%, 88.8% and 100%, respectively. Forty (89%) patients had adequate stem cell collection post-RAD induction (medianSD = 8.94±6.50 x10^8/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 Ang-1/Anng-2, bALP and P1NP compared to 30 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), P1NP (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 year, the 12-month PFS rate and OS rates are high, patients with an ORR of approximately 67% pre- and 84% post-ASCT. With a median follow-up of >1.5 year, the 12-month PFS rate and OS rates are high, 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 OS rates at 12 months were 88.6%, 88.6% and 100%, respectively. Patients 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 comparing 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 (0.7-20.5) (p<0.001) from 1st to 2nd line tx and 11.2 months (0.3-121.9) vs 6.3 months (1.4-11.6) from 2nd to 3rd line tx (p=0.026). The early mortality (within the first year) was 5.9% (3/152), in details only 1/258 of those eligible to ASCT (0.4%) and 3/267 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 complaint neurological, 20% gastrointestinal and 18.2% hematologic toxicity; with Len-based tx 36.4% infective events and 29.8% 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 in median OS noted 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.

**Table 1.**

**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 30 years: conventional chemotherapy (CT) has been gradually abandoned and autologous stem cell transplantation (ASCT), proteasome inhibitors as Bortezomib (Bor) and immunomodulatory drugs as Thalidomide and Lenalidomide (Len) have become the new actors in MM treatment (Tx).

**Aims:** 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 tx to disease progression or last-follow-up. The effect of variables on OS and PFS was evaluated by log-rank test.

**Table 1.**

**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 IMiDs treatment are yet known. While the identification of the IMiDs action via cerebrol (CRBN), Ikarios (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, IRF4 and MYC expressions 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 0-3 scale ranging from 0 (no expression) to 3 as 30% positive cells. Associations between studied proteins’ expression and clinical parameters were assessed using Fisher’s Exact Test for categoric variables and Mann-Whitney-Wilcoxon Test U 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- risk cytogenetic abnormalities (p=0.02) was observed. Furthermore, a trend (p=0.06) was found between 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 BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER ASCT AND MINIMAL RESIDUAL DISEASE (MRD)

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.

Results: 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: 35.5–70.6%) 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.

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

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.

Summary/Conclusions: Our results suggest that tandem ASCT is a very effective treatment modality that can partially substitute for the absence of expensive novel agents with low long-term and lethal toxicities. Tandem ASCT seems to result in superior OS and PFS in comparison to single or salvage second ASCT. More than 10% of patients treated with tandem ASCT experience very prolonged PFS.

E1297
EXTRAMEDULLARY DISEASE IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION: CLINICAL IMPACT IN DIAGNOSIS, TREATMENT AND OUTCOME

Background: Extramedullary disease (EMD) is defined as an infiltrate of clonal plasma cells outside of the bone marrow. The presence of EMD in multiple myeloma (MM) patients (pts) at diagnosis is a relatively uncommon presentation and accounts for about 13% (6-20%) of MM pts. Although several studies...
showed an association of EMD with other adverse prognosis factors and unfavourable outcomes, reports evaluating EMD and outcomes in pts undergoing autologous hematopoietic stem cell transplantation (aHSCT) are scarce.

Aims: We aimed to evaluate the clinical and laboratorial 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, 63% of the most common subtype was IgGκ (45%). In our cohort, 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 (53 vs 36%; p=0.003), vs without (50 vs 36%; p=0.006). EMD was observed in pts with lower International Staging System scores (I and II vs III) (82 vs 64%; 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 (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 aHSCT (3.1 vs 2.4; 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 was no difference between them (p=NS). The median PFS was 51.3 months for global cohort, with no differences seen between pts with and without EMD (50.2 vs 54.1; p=NS). Pts with EMD treated with a PI (57%) presented a higher OS (NR vs 32.5 months, p=0.04), but with no impact in PFS (p=NS), 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 a 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.
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 for ASCIT 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 III 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).

Figure 1.

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.

E1300

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)=[SXRd(t) - SRd(t)] / SRd(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.

E1301

POMALIDOMIDE WITH LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A PROSPECTIVE ANALYSIS IN A POPULATION-BASED REGISTRY

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Background: Patients with relapsed and/or refractory multiple myeloma (R/RMM) have limited treatment options and a poor prognosis. Previous trials showed that pomalidomide combined with low-dose dexamethasone is effective in these patients with improvement in response and survival. These studies led to the approval of pomalidomide as third line treatment in patients with RRMM. A subsequent prospective analysis in a population-based registry was conducted to assess response and survival in patients with R/RMM treated with a pomalidomide-based regimen. Also, we defined subgroups who benefit most of this treatment regimen.

Methods: Patients were eligible for pomalidomide if they received ≥2 prior lines of therapy including bortezomib, lenalidomide and alkylator therapy and developed progressive disease on their last therapy. This is a prospective analysis of patients registered at the nationwide Netherlands Cancer Registry. Treatment consisted of 4mg pomalidomide, day 1-21, combined with corticosteroids. Treatment was discontinued in case of progressive disease or unacceptable toxicity. Primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS), overall response rate (ORR), toxicity, response per risk group (based on cytogenetics and ISS at initial diagnosis) and response per age group (>65 vs ≥65 years).
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 36% and 4% 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 PFS of 5.7 months (95% CI 2.9-8.0) versus 2.8 months (95% CI 1.9-6.6) in patients ≤65, however, this was not statistically significant (p=0.427) (figure 1). For patients achieving ≥PR, median PFS was 9.6 months (95% CI 5.7-Not reached [NR]). Median PFS in patients diagnosed more than ten years prior to initiation of pomalidomide treatment was 9.6 months (95% CI 5.7-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.

Summary/Conclusions: In this analysis the experience in clinical practice of patients with RRMM treated with a pomalidomide-based regimen is reported. These data support results shown in clinical trials. Preliminary data presented here suggest that older patients and patients with a long interval between initial diagnosis and pomalidomide treatment (indicating a less aggressive multiple myeloma) may benefit from this treatment.

E1302

INVOLVED/UNINVOLVED HEAVY/LIGHT CHAIN INDEX CAN PREDICT PROGRESSION IN MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM TRANSPLANT. PRELIMINARY EXPERIENCE

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Background: High-dose therapy followed by autologous peripheral blood stem transplant (APBSCT) has demonstrated to improve overall survival and progression-free survival with a high complete remission rate in multiple myeloma (MM) patients. However, most patients eventually present progression or relapse (P/R). Detection of P/R is mainly based on a significant increase of 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 therapy.

Aims: The aim of our study was to evaluate the potential role of the Involved/Uninvolved Heavy/Light Chain index (I/Ui) in this setting.

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 APBSC. Relapse or progression was defined according IMWG criteria. To identify factors that predict disease progression in MM transplanted patients, we studied heavy/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 (HHL) was calculated as IgGk/IgGλ or IgAk/IgAl 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 of the monoclonal protein. Regarding IgA MM, we established a cut-off value of 2.0 for I/Ui that allowed the discrimination of patients at high risk of early progression (values above 2.0) from those in CR, whose levels of I/Ui are always below 2.0 (p=0.02).

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 S-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 CR showed significantly longer PFS with median 42 months than those in less than CR with PFS median 29 months (p=0.0196, log-rank test). This was associated with an increased hazard ratio of relapse (0.3565) in CR group.

Summary/Conclusions: Achieving immunophenotypic CR is clearly associated with longer progression free survival compared to conventional CR. Reaching CR should be a goal of myeloma treatment.

E1304
REGULATION OF NORMAL AND MONOCLONAL IMMUNOGLOBLIN 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 quantified in 50% of CLL cases. Microenvironmental factors, such as soluble Syndecan-1 (synd-1) and BlyS normally promote lymphoplasmacytic differentiation as well as their secretory activity, whereas other cytokines, like TGF-beta1, inhibit it. Determination of Ig is necessary in MM and WM for diagnostic purposes and for monitoring patients, while in CLL, sFLC has prognostic value. The total amount of secreted Ig does not really reflect disease burden. The heavy chain Ig can be accurately determined with the Heavyvite method (that measures separately HLC-IgA, -G, -M kappa or lambda), thus allowing exact quantification of the amount of pure monoclonal fraction but also the degree of suppression of polyclonal Ig, both being reflected by the corresponding ratios (HLCR).

Table 1.

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Aims: To determine any possible relationship between the amount of IgG secreted by the B-cells and TGFBeta1, as well as with disease outcome.

Methods: We studied 269 patients: 105 with MM (79 IgG and 26 IgA, of whom 33%, 31%, and 36% were staged 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 from diagnosis to last visit or death (median follow up to 5 months). sFLC/sFLC and HLC/HLCR were determined by nephelometry (Freelite™ and Heavylite™, 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 disease outcome. By inhibiting both monoclonal and polyclonal Ig, TGFbeta1 correlated in MM with 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 TRANSPLANT (APBSCT) EXPRESS A DISTINCTIVE IMMUNE PROFILE WITH POTENTIAL PROGNOSTIC 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 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, in order to confirm their specific immune signature, and also in the duration of complete remission (MM-LTCR) after autologous transplant (APBSCT). The research was performed in the comprehensive 8-color flow cytometry panel. Populations of CD4+ and CD8+ T-cells from PB were quantified, including naive, central and effector memory T-cells, as well as subpopulations of B-cells: naive, 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.

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 Mabeian follow-up. In sCR, no patients were found when compared to HA. However, naive B-cells (CD19+CD10−CD21+IgM−) proportion was higher in patients and a corresponding reduction of marginal zone-like B-cells (CD21+CD23−CD10−CD19+) and plasmablasts (CD20+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 naive T-cells and an increased percentage of effecter T cells, which probably exert a competent immune surveillance. Conversely, the increase in naïve B cells may guarantee the humoral response host immunity, inducing survival of the individual 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.

Study partially performed with research grants from the spanish Leukemia and Lymphoma Foundation and Grant PI12/00494P from the Fondo de Investigaciones Sanitarias and FEDER funds.

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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Background: The success of bortezomib and lenalidomide in improving outcomes as first-line therapies in multiple myeloma (MM) patients has been achieved at a very high cost. Treatment has become difficult to access for patients living in low to middle-income countries, as most receive assistance by public healthcare systems wherein novel drugs are unaffordable.

Aims: To compare the outcomes of MM patients who can afford private insurance and treatment in a private center (PrivC), with those managed in a public center (PubC), who do not have access to healthcare coverage and are treated on an out-of-pocket basis.

Methods: We analyzed records of 148 patients diagnosed with MM in two health sectors in Monterrey, Mexico, from October 2007 to July 2016; 77 (52%) from PubC, where the most common induction therapy was cyclophosphamide, thalidomide-dexamethasone, followed by thalidomide maintenance, and 71 (48%) from PrivC wherein bortezomib or lenalidomide-based induction and lenalidomide maintenance were used. We compared demographics, disease stage, response rate and survival among both groups.

Results: Median age, gender and frequency of immunoglobulin isotype did not differ significantly between the two groups. Patients treated in PubC were more likely to be diagnosed with advanced stage disease (ISS III 42% vs 26% p<0.05). Median follow-up was 36 months (range 3-120 months). Autologous transplantation was performed in 80% of the transplantation-eligible patients in PrivC and only in 31% of PubC. At least a very good partial response to induction therapy was achieved more often in the PrivC among transplantation-eligible (65% vs. 42%, p<0.05) and ineligible patients (66% vs. 41%, p<0.05). Overall survival was significantly higher in PrivC for transplantation-eligible (median 84 months vs 42 months, p<0.05). After controlling for disease stage and transplantation factors, the risk of mortality was still higher in PubC (HR 1.49; 95% CI:1.0-2.2, p<0.05).

Summary/Conclusions: Stage at diagnosis, induction therapy and autologous stem cell transplantation were contributors to survival disparities between patients treated in public vs private health care facilities in Mexico. These findings underscore the need for more efforts to improve the affordability of novel agents and transplantation settings in public health services.

Myeloproliferative neoplasms • Biology

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 being 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 Flu-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 cells harboring CALR type1 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 of 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 the advancement in the use of JAK2 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. Hexa-bromocyclhexane 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 signaling in HEL cells as well as in granulocytes of JAK2V617F positive ET and PMF. Absence of JAK2V617 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 Hexa-bromocyclohexane 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 DISRUPTED: A NOVEL LIQUID BIOPSY TOOL OF RESPONSE TO RUXOLITINIB?
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Background: Microparticles (MPs) are small vesicles (0.1-1 micron) deriving from plasma membrane budding during homeostasis and cell activation. MPs express antigens and contain constituents from cell of origin and are increased in conditions that are characterized by high cell turnover or death, particularly inflammatory, autoimmune and neoplastic diseases. Myelofibrosis (MF) is a clonal neoplasia of the hematopoietic stem/progenitor cells characterized by diffuse stromal fibrosis, dysplasia of megakaryocytes (MK) development and platelet (PLT) activation. Mutations in 3 genes (JAK2, CALR, MPL) and chronic inflammation are the main pathogenic drivers of MF. Ruxolitinib (RUX), a JAK1/2 inhibitor, suppresses both clonal myeloproliferation and release of proinflammatory cytokines, reducing splenomegaly and constitutional symptoms in around 50% of patients (pts). We hypothesized that MPs, as mediators of inflammation, could be overexpressed in MF and possibly predict responses to RUX.

Aims: This study aims to: 1) enumerate circulating MK and PLT-derived MPs of MF pts; 2) evaluate the effect of RUX on MPs production by PLT and MK; 3) investigate whether circulating MK and PLT- MPs may be a biomarker of response to RUX.

Methods: EDTA-anticoagulated peripheral blood from healthy donors (HD, n=10) and JAK2V617F positive MF pts (n=12) at intermediate-2 high IPSS risk was collected at baseline and 3 and 6 months after RUX therapy and immediately centrifuged. Microparticles in megakaryocytes (MK) development and platelet (PLT) activation. Mutations in 3 genes (JAK2, CALR, MPL) and chronic inflammation are the main pathogenic drivers of MF. Ruxolitinib (RUX), a JAK1/2 inhibitor, suppresses both clonal myeloproliferation and release of proinflammatory cytokines, reducing splenomegaly and constitutional symptoms in around 50% of patients (pts). We hypothesized that MPs, as mediators of inflammation, could be overexpressed in MF and possibly predict responses to RUX.

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 express different surface marker expression patterns as compared to the other ET groups and HD. On the other hand, JAK2 V617F and CALR type 1 ET platelets exhibited normal to increased expression density of these receptors as compared to HD. Variable patterns were observed amongst the other ET genetic subgroups, with reduced responses especially upon challenge with Aggretin A or collagen, while platelets from the JAK2 ET subpopulation displayed reduced reactivity to collagen and the other ET groups. This study aimed to establish a better diagnosis/prognosis of the disease.

Summary/Conclusions: These preliminary results suggest that MPL S204F/P platelets are intrinsically defective (hypo-reactive), in contrast to JAK2 V617F platelets (hyper-reactive), while in other genetic subgroups, potential defects are most probably synergistic and/or acquired by treatment. Data suggests that JAK2 V617F and CALR type 1 platelets could also undergo basal degranulation or vesiculation in the circulation. Analysis of the platelets has identified characteristics in different genetic groups of ET that should be further investigated to account the different treatment conditions and using larger cohorts of patients. When specific functional and phenotypic platelet patterns are established it 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). None of the currently available peripheral blood and bone marrow cytogenetic panels (Illumina) are designed to efficiently screen 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: To characterize genomic aberrations in PMF using SNP-A and NGS methods.

Methods: PMF peripheral blood samples were screened by Infinium HD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChips (Illumina Inc., CA). NGS analysis was performed using TruSight Myeloid 54 gene panel (Illumina) and single nucleotide polymorphism array (SNP-A) methods and single nucleotide polymorphism array (SNP-A) methods and single nucleotide polymorphism array (SNP-A) methods and single nucleotide polymorphism array (SNP-A) methods and single nucleotide polymorphism array (SNP-A) methods as 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 this study. SNP-A 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: 5p LOH (55.7%), 20q deletion (11.5%), 1q duplication (4.9%), 19p deletion (3.2%), 1q LOH (3.2%), 6q LOH (3.2%). NGS analysis detected 219 gene mutations (in a total of 27 genes) in 108 patients (98%). The most frequently mutated genes were: JAK2 (62.9%), CALR (27.8%), ASXL1 (20.3%), TET2 (16.6%), MPL (7.4%), <5% ZRSR2, EZH2, DNMT3A, U2AF1, ET16, SF3B1, IDH1, IDH2. Recurrent specific mutations were identified in 10 genes. 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”. Interestingly, out of the 267 BCR-ABL1 mutated cases the ratio was equal. Most of the patients (18/19) had one mutation with the higher load, whereas in 4 cases were present in 19/1775 (1%) cases:

**Summary/Conclusions:** A number of associations between gene mutations and chromosomal aberrations was revealed in PMF. Co-presence of 9p LOH with JAK2V617F 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 5545 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 9x10^9/L, hemoglobin level (Hb) was 15g/dl and platelet count was 329x10^9/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 Va117Phe (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 (3%). 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 (0.1%). The distribution of the 267 cases was similar to the genetic distribution in the whole cohort. However, the Kaplan–Meier method followed by sequencing of probably mutated samples. Karyotype research was done for 48 (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 (CNH) unfavorable CA (85% of DIPSS adverse muta-

tions), 1q deletions, 20q deletions and CALR(+)ASXL1 wide type (wt) pts seem to have better OS than CALR(-)ASXL1 wt (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

**E1313**

**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 ASXL1, EZH2, IDH1/2 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 ASXL1, EZH2, IDH1/2 genes with high resolution melt- ing method followed by sequencing of probably mutated samples. Karyotype research was done for 48 (43.6%) pts.

**Summary/Conclusions:** One-third of the cases can be diagnosed having more than one mutation according to the detection of BCR-ABL1, CALR and/or MPL mutation in an unselected cohort with suspected MPN. The frequency of double mutated JAK2, CALR and MPL cases is 1%. In CML cases BCR-ABL1 fusion and JAK2 or CALR mutation were detected in 2% of the patients. Impact of these parallel genetic events on the clinical course of the disease has to be evaluated in the future.
was 0.9 years in TN/ASXL1mut. 3.6 years in TNASXL1wt, 13.8 years in DM(+)ASXL1wt and was not reached in DM(+)ASXL1mut (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 (p=0.007) but not for DM(+) group (p=0.788). The better OS was observed in ASXL1 wt pts with low risk (LR) karyotype (Me 6.4 years, p=0.0058). There were no differences in OS of ASXL1 wt- HR, ASXL1mut-LR and ASXL1mut-HR pts (1.4 vs 1.6 vs 1.2 years, p=0.493).

Summary/Conclusions: The differences in OS were more statistically relevant in groups divided by TN/ASXL1 and karyotype/ASXL1 status. The presence of ASXL1mut significantly worsens OS in the TN group. In pts with in any of the findings: HR karyotype or ASXL1mut – was significantly shorter than in cytogenetically favorable ASXL1wt counterparts.

E1314

JAK2 HAPLOTYPE 46/1 (GGCC) HAS NO EFFECT ON THE PRIMARY RISK OF JAK2 V617F MUTATION, BUT IT STRONGLY POTENTIATES THE PROGRESSION OF GROWN ALLELE BURDEN IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Several research groups have determined that the JAK2 46/1 (GGCC) haplotype in multiple ethnic groups is strongly associated with a predisposition 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-Larran A et al. Leukemia Research 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 [Krichievsky S et al. 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 allelic 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 MPN, 17 patients were treated with hydroxyurea and 20 were treated 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 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 significantly shorter than in cytogenetically favorable ASXL1wt counterparts.

Summary/Conclusions: No significant differences of the carrier haplotype JAK2V617F allele burden between control group and patients with minimal allelic burden (less than 5%) JAK2 2V617F have been observed. This is evidence against primary "hypermutability" hypothesis. A further increase in allelic load is more pronounced in carriers of haplotype 46/1 that supports the "fertile ground" hypothesis. We hypothesize that DNA mutation JAK2V617F repair is down-regulated in 46/1 haplotype carriers.

E1315

MINIMAL RESIDUAL DISEASE MONITORING BY DIGITAL PCR FOR JAK2V617F DETECTION IN PATIENTS WITH MYELOFIBROSIS (MF) OR ACUTE MYELOID LEUKEMIA SECONDARY TO MF AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Myelofibrosis (MF) is one of the BCR-ABL1-negative Chronic Myeloproliferative Neoplasms (MPNs), characterized by clonal expansion of abnormal hematopoietic progenitors and gradual replacement of normal bone marrow with fibrous tissue. MF patients' prognosis is widely variable and the impact of radiation can vary between patients to many extent. At present, Allogeneic Stem Cell Transplantation (ASCT) is the only curative treatment option for these patients. The most frequent phenotype-driving mutation in MF is the V617F mutation in the JAK2 gene. A high sensitive quantification of JAK2V617F mutation load can be useful to assess Minimal Residual Disease (MRD) in treatment 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.

Aims: 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).

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 evaluated 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 mutation load (up to 0.006%), confirming to be much more sensitive than ASO-PCR (0.5%). In 4 patients, early after transplantation, we observed by ddPCR a low level of MRD that progressively increased during the follow-up and anticipated a decrease in donor chimerism level and a worsening of clinical situation. In 2 patients, who showed a full donor chimerism and complete hematologic remission of the disease, very low levels of MRD (ranging 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 both by a high level of sensitivity and specificity.

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 s-AML and s-AML. 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
E1316
S100A8/9 ACTIVATION OF MAPK PATHWAY IS SUPPORTED BY ITS RECEPTORS RAGE AND TLRI4 IN POLYCYTHEMIA 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 thrombocytopenia (ET), primary myelofibrosis (PMF), according to JAK2V617F and calreticulin (CALR) mutation status.

Methods: S100A8/9 factor is examined in granulocytes of MPN using immunoassay to evaluate the concentration 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+, JAK2V617F-/CALR+, and JAK2V617F-/CALR- for ET and PMF.

Results: S100A8/A9 proteins 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- PMFs, partially prevented by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated MAPK activation has been significantly augmented by S100A8/9 in the absence of CALR 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 S100A8/9 has been prevented by TLR4 and RAGE inhibitor in PV patients. S100A8/9 stimulated granulocyte cycle arrest in G2M phase has been stopped by JAK1/2 inhibition.

E1318
TTC GAMA CLONALITY ASSESSED BY NGS DOES NOT HELP TO DISinguish EGPA FROM HES
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Background: Hypereosinophilia-associated 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) are related to several overlapping clinical and laboratory features, making their differentiation challenging in both diagnosis and treatment. The common feature shared by EGPA is a significant T cell receptor gamma repertoire in EGPA patients, whereas the presence of CALR mutation augmented S100A8/9 factor, further on ameliorated by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated MAPK activation in JAK2V617F-/CALR+ ET. In contrast, S100A8/9 mediated MAPK activation in 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- PMFs, partially prevented by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated MAPK activation has been significantly augmented by S100A8/9 in the absence of CALR 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 S100A8/9 has been prevented by TLR4 and RAGE inhibitor in PV patients. S100A8/9 stimulated granulocyte cycle arrest in G2M phase has been stopped by JAK1/2 inhibition.

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 (qPCR) 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 diagnosis, 3 patients suffered a thrombotic event after diagnosis, 1 patient had suffered transformation to AML. Median Hb, WBC and platelets at diagnosis were respectively 14.75g/dl, 8.85 x10 9/L and 720x10 9/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 t-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-synchronous 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 (45.5%) had 1 mutation, the other 3 samples presented no or more mutations (13.6%). TET2 was the most frequently mutated gene (18.2% of patients, mean allele frequency of 24.45%), followed by JAK2 (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%), KIT (4.5%), BCL (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 MPN as primary myelofibrosis.
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 measuring 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

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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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Background: Quality of life (QOL) is a critical aspect of cancer treatment and survival. A strong association exists between QOL and overall survival (OS) for numerous malignancies including breast, gastro-esophageal, colorectal, lung, prostate, ovarian, and head and neck cancer (Sloan 2012, Montazeri 2009, Nilsen 2017). Healthcare organizations have used symptom burden as a primary therapeutic endpoint when assessing the benefit of JAK inhibitors in myelofibrosis (MF) in clinical trials, although QOL was also considered. To date, little is known about the association of these items in regards to overall survival in MF.

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 SF-36 and EORTC QLQ-C30 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 OS were similar to previous published results (Verstovsek 2015). Symptom Burden: When comparing OS by TSS quartiles at baseline, no significant differences 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 MF-SAFv2.0 high risk pts demonstrated a significant survival benefit from symptom burden (scored worst) on OS. Notably, no other variable was found to impact survival (Verstovsek 2015). Consistent with previous published results, OS by TSS quartiles at baseline, we 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]).

E1322
CHARACTERIZATION OF DISEASE AND OUTCOMES OF PATIENTS WITH MYELOFIBROSIS: A POPULATION BASED STUDY

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm with profound negative effects on health related quality of life and survival. It is characterized by clonal myeloproliferation, ineffective erythropoiesis, bone marrow stromal changes, hepatosplenic extramedullary hematopoiesis, and aberrant cytokine expression. Although progress has been made in the understanding of the pathogenesis and management of MF, there are still unresolved issues regarding prognosis and causes of death.

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.

Figure 1.
Results: The final study cohort comprised of 2,619 PMF pts. Median follow up period was 28 months (interquartile range 9-57 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), rouxotubin 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 (<0.01) and platelet count (<0.01), and negatively correlated with age (<0.01), peripheral blast percentage (<0.03), ferritin (<0.01), prognostic scoring models (<0.01 for IPSS, DIPPS and DIPSS+) and pack-year smoking history (<0.01). SA did not correlate with spleen size or any specific somatic mutation, but negatively correlated with somatic mutation burden (<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 (in months) of 9.34, 25.3, 48.4, and undefined 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 scoring (IPSS, DIPSS, DIPSS+) and comorbidities. For PNA/N, SA remained significant when controlling for IPSS and DIPSS (p<0.05). On univariate analysis, the influence of SA on OS remained significant after controlling for prognostic scoring (IPSS, DIPSS, DIPSS+) and comorbidities. SA was positively correlated with inferior PFS (<0.01) and OS (<0.01). 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, its independent prognostic value incorporated into pre-existing prognostic models increased the accuracy of prediction. Its 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 indices, 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 2015 and December 2016 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. Repertoire was limited to high quality exon nonsynonymous, intrinsic 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: 179 patients fulfilled the WHO diagnostic criteria for MPN: 107 were diagnosed with myelofibrosis (MF), 26 with polycythemia vera (PV), 21 with essential thrombocytosis (ET), 11 with other MPN, 15 with unclassifiable and 12 with MPN/AML. In 6 patients with ‘triple negative’ MPN, who lacked mutations in the driver genes JAK2, CALR or 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 risk by IPSS (R1) profile based risk scoring at initial presentation in a cohort of 350, 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 12/2017.
inhibitor (JAKi) therapy. All high-risk, transplant-eligible MF patients were considered for transplantation irrespective of their HMR status. Nine patients with low/intermediate-1 risk MF bearing HMR mutations were considered for a clinical 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 with IDH 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 characterization of triple 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 should be a part of the standard of care in MF, and in the investigation of triple 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 Hematology 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 cell count, spleen ≥10cm, marrow fibrosis grading, time from MF diagnosis 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 Centers. At RUX start, median age was 67.5 years (range 35.6-89.0) with a male prevalence (57.1%); International Prognostic Score System (IPSS) was intermed evaluable-1 (16.0%), intm-2 (47.5%), high (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 0 in 105 pts (30.6%), one in 74 pts (21.6%), two in 58 pts (16.9%) and ≥ 3 in 106 pts (30.9%). Median RUX exposure was 21.2 months (3-56.2). In multivariable Cox regression analysis, factors negatively correlating with OS from RUX start were: transfusion dependency (HR: 2.65; p<0.001), CCI ≥3 (HR: 1.67; p<0.001), BMI<21 (HR: 1.74; p=0.039), and IPSS (intm-2: HR: 3.19; p=0.057, high risk: HR: 6.8; p<0.001). CCI ≥3 was an independent predictor of OS (p=0.001). Scoring values were assigned to OS at 3 years was 91.8%, 65.6% and 34.8% in group 1, 2 and 3, respectively (log rank p<0.001) for a median OS of undefined, 66.7 and 22.8 months. Notably, while 88.7% of high IPSS risk pts clustered in group3, only 60.5% of pts in group1 were at intm-2 IPSS risk, and 48.6% of pts in group2 were at high IPSS risk. The achievement of a spleen response at 6 months (39.2% vs 36.4%, p=0.71) was not influenced by lower BMI. However, pts achieving a spleen response at 6 months had 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 may influence survival in RUX-treated MF pts. Taking into account these additional 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 thrombocytethemia (pET)-MF and post polycythemia vera (pPV)-MF are considered rare diseases associated with significant morbidity. Diagnostics and therapeutic options have significantly improved during the last decade by development of novel drugs, improvement of allogeneic stem cell transplantation (SCT) procedures and supportive care. Whereas the characteristics of PMF, pET-MF and pPV-MF patients (pts) participating in clinical trials are well analyzed, data are rare for the general MF population including patients not included in or eligible 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, 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

544 | haematologica | 2017; 102(s2)
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 as were as follows:<50 years (y) (11%), 50–69y (19%), 70+1y (31%), and >70y (40%). Current age was >65y in 70% of all pts. PMF represented the largest MF cohort (77%), followed by MP-MF (10%), PV-MF (9%) and unspecified (6%). Most pts (92%) had disease <5 years (29% 1–5 years; 1% <1y (15%); unknown (1%). Key current blood values at time of diagnosis included abnormal thrombocyte counts (<500GPT/L; 60%); <100GPT/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 (21%), <8 (8%), unknown for 3% of the pts. Constitutional symptoms were present in 20% of the pts. Common symptoms included splenomegaly (60%), decreased fitness (41%) and weight loss (16%). Pruritus was present in 5% and night sweats in 9% of all pts. An individual Dynamic Prognostic Scoring System (DIPSS) score was calculable in 495 pts: 19% low risk, 52% intermediate-1, 20% intermediate-2 and 5% high risk disease. Concomitant ischaemic diseases were common, most often cardiac (56%). Most common medical treatments included cytostatic (37%), anticoagulation (25%), JAK-inhibitors (23%) and none (24%). Non-medical treatments were rare: stem cell transplantation (3%), splenectomy (2%) and spleen irradiation (3%). Only 31% of all pts received red blood cell transfusions, however 7% had received >90 units.

Summary/Conclusions: Daily practice MF pts share several characteristics with MF trial cohorts (e.g. COMFORT). As expected the diseases were not as 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.
Table 1.

<table>
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<th>Soluble selectin, µg/ml (assay)</th>
<th>HSPB1 (±SD)</th>
<th>p</th>
<th>ASP (±SD)</th>
<th>p</th>
<th>HSPB1/ASP (±SD)</th>
<th>p</th>
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<td></td>
<td></td>
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<tr>
<td>12-C5(B2)</td>
<td>65.4±11.3</td>
<td>0.025</td>
<td>44.1±11.8</td>
<td>0.01</td>
<td>2.03±1.1</td>
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<tr>
<td>12-C5(B3)</td>
<td>105.8±20.3</td>
<td>0.028</td>
<td>107.1±20.2</td>
<td>0.03</td>
<td>1.00±1.2</td>
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<tr>
<td>12-C5(B4)</td>
<td>65.9±11.5</td>
<td>0.007</td>
<td>335.2±26.7</td>
<td>0.00</td>
<td>0.19±0.01</td>
<td>0.001</td>
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</table>

**Figure 1.**

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 led 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

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 Kusak-Walls one way analysis of variance, The Mann Whitney U test, the Chi squared test, and 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=0.001); 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=0.034) 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).

E1330

NON-DRIVER MUTATIONS IDENTIFIED BY A 190-GENE NEXT GENERATION SEQUENCING PANEL IN PATIENTS WITH PRIMARY MYELOFIBROSIS AND POST-POLYCYTHAEMIC/ESSENTIAL THROMOCYTHAEMIA

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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 mutation. 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 JAK2/V617F/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=0.085) and PMF patients with vs. without driver mutations (3 vs. 3.18; P=0.688). 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.
ERITHROPOIESIS 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 causes 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 et al. in 2016 (Cervantes F et al. Blood 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. S. 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 received epoetin after ruxolitinib start for persisting or worsening of anemia. In particular, 5 were already RCA 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 time to response and median ESA start was 8.5 months (range 2.5-10). ESA dose was reduced in 31 patients (96.9%) and ESA was stopped in 1 patient (3,1%) due to RCA transfusion dependent. Median basal endogenous erythropoietin level was 58 U/I (range 8-146 U/I). Overall response rate was 87,6%, with 68,8% of erythroid response and 18,8% of partial response. Median time to response and median 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 16.6 (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 QLG-C30 data at baseline and week 24, 51 (23%) patients had deterioration, 81 (47%) were stable and 110 (50%) had improvement based on QLG-C30 GH/QOL changes. Mean (95% CI) changes in TSS for the three groups were 0.8 (-2.5 to 4.2), -1.4 (-3.6 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 the predicted change in TSS, 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.
response duration were 4 and 31 months respectively. 23% of patients lost response after a median time of 16 months. Seventy-five-fold patients responded to ruxolitinib in terms of spleen size, of whom 86.4% also achieved an erythropoietin response to ESA. A spleen increase during ESA treatment in patients responding to ruxolitinib was observed in 2 patients only.

No thrombotic events and no toxicity were reported over treatment with ESA. Success was effective in improving anemia in MF patients treated with ruxolitinib. We observed a high response rate in this patients series without significant toxicities. In particular no thrombotic event e no negative impact on response to ruxolitinib was reported. This results may be partially explained by the selection of patients with endogenous erythropoietin level below 250 U/I, but they could also suggest synergistic activity of ESA and ruxolitinib.

**E1334**

**COMPARING THE SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS (PTS) WITH DIPSS 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 splenomegaly and symptoms and increased overall survival in pts with intermediate (Int)-2– and high-risk MF by the International 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 (>9 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 (≤1%, 18%, 44%, 84.5%). 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%, 56%, 45%). Reasons for treatment discontinuation included adverse events (AEs; 15%, 27%, 31%), 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 ≥25% of pts were pneumonia (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 (2.7-75 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 44%, and ≥50% 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%.

**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.
Methods: In pts with MF.

Aims: Of a subset of JUMP pts provides information on this approach. Up titrating may reduce the risk of cytopenia development. An ad hoc from clinical practice suggests that starting RUX at 10 mg bid and subsequently 200×10^9/L, >200×10^9/L, respectively). Although not per protocol, some pts started RUX at 10mg bid. The primary endpoint was safety. Secondary end points included changes in spleen length and symptoms.

Results: A total of 48 pts (primary MF, 60%) started RUX at 10 mg 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, 351×10^9/L (<100×10^9/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 25.8 mg/day (SD, 10.1) and was comparable to those (33.2 and 23.3 mg/day) of patients starting at higher doses (20 and 15 mg bid; all due to AEs). A total of 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). Mean duration of the disease until 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-alpha (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, and 60 pts (50%) experienced significant improvements in symptoms. From wk 4 to 48, 43%-59% and 45%-68% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACIT Fatigue, respectively.

Summary/Conclusions: A small cohort of pts in JUMP started at 10 mg 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 (NCT02966353).

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 until 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-alpha (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, and 60 pts (50%) experienced significant improvements in symptoms. From wk 4 to 48, 43%-59% and 45%-68% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACIT Fatigue, respectively.

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E1336 THE NEGATIVE PROGNOSTIC IMPACT OF BASOPHILIA, EOSINOPHILIA AND MONOCYTOSIS AT DIAGNOSIS IN PRIMARY MYELOFIBROSIS

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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-

Figure 1.
positive MPNs, where they associate with an accelerated phase of disease, and seem to correlate with worse survival in myeloid dysplastic 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 fulfilled WHO 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) >1.5 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 cohort, a new 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 27.3 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 7.3 months, compared to 43.8 months for patients under the cut-off. A total of 30.9% of patients had basophilia at diagnosis, with no differences according to gender or age. The median OS in patients with basophilia was 25.6 months, and 32.5 months in patients without. With a new cut-off of 0.25 G/L of basophils, with a specificity of 88.9% (95% CI: 70.8-97.64%); 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-off, this difference in OS increased to 79.9 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.
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. JAK2 V617F positive (n=101; p<0.0005), JAK2 V617F negative (n=34/100) 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 hydroxyurea, busulphan or IFN (8 of these 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 found that those patients with either current or prior exposure 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 shorter 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 JAK2 V617F 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 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. 2011;70(10):2548). Both hypcholesterolemia (p<0.001) and weight loss>10% (p<0.001) have been associated with decreased survival in PMF patients (Mesa et al. Blood 2009 114:3918). JAK 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. At baseline, the average BMI 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 leptin 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=0.001) and most correlations were observed between baseline and final BMI 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=0.01). Ruxolitinib: Most correlations with nutritional and metabolic markers mirrored with baseline scores (Figure 1b). For ruxolitinib-treated patients, changes in JAK2V617F mutation status inversely correlated with changes in serum cholesterol (-0.26, p=0.008), leptin (-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).

Figure 1.

Summary/Conclusions: Nutrition decline remains an unmet need for many MF patients. JAK2 inhibition represents a potential source to improve symptom burden in those who qualify for therapy. Leptin closely correlated with many other nutritional values suggesting this may be a good marker of nutritional status in PMF patients. CRP was inversely correlated with BMI, suggesting the importance of inflammation as a contributor to weight loss. Further study into the unique nutritional needs of myelofibrosis patients is warranted.

E1340


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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
01/01/2006 to 31/12/2009 (385 patients), with a median follow-up of 58.2 months.

Methods: The characteristics of two groups of patients are reported in the Table 1. No differences could be found between the two groups according age, gender, platelet and WBC count and Hb level. Cardio-Vascular Risk Factors (CVRF), spleen enlargement and the occurrence of previous thrombotic events. The frequency of the JAK-2 V617F mutation resulted significantly different (49.1% vs 68.4%) but in the group I the search of the mutation was never performed at the diagnosis. TFS and OS were calculated from the date of diagnosis of ET to the date of event with Kaplan-Meier product limit method; the comparison of proportions and median values was computed with the Chi-squared and the Mann-Withney tests, as indicated.

Results: No significant differences emerged neither for TFS (p= 0.09, HR 1.42, 95% C.I. 0.89-2.30) nor for OS (p= 0.15, HR 1.34, 95% C.I. 0.87-2.06). We also evaluated the type of treatment used in the two groups to assess the potential link between the therapy and TFS or OS (Table 2). No difference emerged between the two groups as for anti-aggregating (mainly ASA), equally utilized in both groups, 287/369, 77.8%, and 330/383, 78.3%, respectively (p=0.95). As for the cytoreductive therapy, Hydroxyurea was used in 74.8% vs 67.9% (p= 0.60) and alkylating agents in 1.9% vs 2.1% (p= 0.85), whereas the Anagrelide resulted utilized in 10.6% vs 3.9% (p= 0.001) and Interferon in 9.5% vs 5.2% (p= 0.037), respectively. The more frequent use of Anagrelide and Interferon in the first group (2000-2005) didn’t modify the prognosis (as for TFS and OS) of the patients.

Summary/Conclusions: Unfortunately, no improvement, neither as the TFS nor the OS was observed (Fig. 1 and 2): more efforts to better identify the groups at risk and, hopefully, the introduction of new drugs as JAK-2 inhibitors could change the prognosis of ET patients.

Figure 1.

E1341

CUTANEOUS INVOLVEMENT IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS—SINGLE-CENTER EXPERIENCE
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Background: Philadelphia-negative chronic myeloproliferative neoplasms (MPNs) may present clinical dermatological manifestations at the time of diagnosis, as well as during the course of the disease. On the other hand, also its 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 xerosis and/or keratosis pilaris (76.2% patients), nail changes (41.3%), acniform 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 photoprotection measures. The results obtained support the recommendation of 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.
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 mU/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 BM 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 leukocytosis criteria outlined in the Carabbi algorithm. The figure. The BM examination was performed on 33 patients who met pre-defined criteria for the timing of bone marrow biopsy. About one third of the 33 patients did not present in the WHO classification for 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 Carabbi algorithm can be used to further discriminate early PMF and early idiopathic myelofibrosis from other 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 PACRITINIB (PAC) IN PATIENTS WITH MYELOFIBROSIS (MF) ON THE PERSIST-2 PHASE 3 TRIAL


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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 who have failed corticosteroids and/or splenectomy. PAC, a new oral inhibitor of JAK1/2/3, appears to translate into an improved benefit/risk profile of PAC BID regimens in pts with MF from the PERSIST-2 trial. These differences appear to translate into an improved benefit/risk profile of PAC vs QD regimens.

Methods: PolYA RNA extraction from MPN-eo cases, RNA-Seq library prepa-

E1344
ZMYM2-F3LT3 IS A RARE, RECURRENT, CYTOGENICALLY CRITICAL FUSION IN MYELOID/LYMPHID 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 fusions have been described, most of which are associated with visible cytogenetic abnormalities. However these fusions are rare, and the pathogenesis of the great majority of patients presenting as myeloproliferative neoplasm 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 prepa-

Summary/Conclusions: As predicted by PK modeling and simulations analy-

Results: Of 20 cases tested by RNAseq analysis, just one cryptic TK fusion was identified: ZMYM2-F3LT3, 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 test whether this might be recurrent, we analysed 105 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 leucocytosis (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-Ida), an alloge- nic PBSCT from an unrelated donor was performed 13 months after diag- nosis. As a consequence of chronic GvHD and septic shock, the patient died 6 months after allogeneic PBSCT. The ZMYM2-F3LT3 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-F3LT3 positivity, treatment with sunitinib was commenced. Blood counts started to improve from day 4 and normalized after 3 weeks. Dur- ing a pause of 3 weeks due to pulmonary infection, leucocytes/eosinophils rap- idly increased, but no PCR recurrence was detected 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. Mechanistic studies of ZMYM2-F3LT3 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.
E1345 COMPLETE HEMATOLOGIC AND CYTOTOGENETIC RESPONSE IN A PATIENT WITH FIBROBLAST GROWTH FACTOR RECEPTOR 1 ACTIVATED MYELOPROLIFERATIVE NEOPASMS RECEIVING INCBO54828 N. J. How, 1 J. E. Spruit, 1 E. Asatiani, 2 S. Varjosovska, 1 M. Bak, 3 T. Jess, 3 E. M. Flachs 3, A. O. Zwillich 3, K. Juel, 5 H. Frederiksen 5, H. C. Hasselbalch 5
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Background: Study reveals 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 estimated the risk of IBD in patients with MPN.

Methods: We used valid Danish national registries, covering more than 5 million inhabitants, and included all patients diagnosed with either essential thrombocythemia (ET), polycythemia vera (PV), myelofibrosis (MF), or unclassifiable myeloproliferative neoplasm (MPN-U) between 1994 and 2013. For each patient, 10 individually age- and sex-matched comparisons were included. Patients and comparisons were followed until first occurrence of any IBD diagnosis (ulcerative colitis or Crohn’s disease), death, emigration or end of 2013. The risk was only calculated if five or more individuals were diagnosed with IBD 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 comparisons was 1.8 (95% CI: 1.7-2.0). 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.

E1346 THE GRADE OF STROMAL CHANGES IMPACTS ON PROGNOSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS U. Gianelli, 1 S. Fiori, 1 D. Cattaneo, 2 A. Bossi, 3 I. Cortinovis, 3 C. Bucelli, 3 N. Orofino, 2 A. Iurlo 2
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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 a bone marrow trephine biopsy was available. The stromal grades 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: Oste-0 in 72 cases, Oste-1 in 24, Oste-2 in 19 and Oste-3 in 7. Patients’ population was composed of 56 males and 66 females (MF=1/1,2) with a median age at diagnosis of 68 years (range 30-85). Clinically, at presentation, anaemia with haemoglobin values less than 10 g/dL was present in 20 (16%) patients, leukocytosis more than 25 x10⁹/L was identifiable in 4 (3%) patients, and platelets count less than 100 x10⁹/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 (38%) 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 primary myelofibrosis, proved to be reproducible and adopted by the updated WHO 2016 classification.

Of the 8,210 MPN patients, 80 individuals were diagnosed with IBD 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 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 THROMBOCYTHEMIA WITH AQUAGENIC PRURITUS: AN ENTITY WITH MORE AGGRESSIVE CLINICAL AND BIOLOGICAL PROFILE AT THE DIAGNOSIS AND A HIGH MORBIDITY DURING THE FOLLOW-UP. C. Le Gall-Ianotto1,2,*, R. Le Calloch3, L.-M. Mollard4, L. Miseri1, J.-C. Ianotto4
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Background: Polycythemia vera (PV) and essential thrombocytosis (ET) are Ph-negative myeloproliferative neoplasms in which arterial or venous thromboses and phenotypic evolutions (leukemia, myelofibrosis) are the most frequent 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 NEOplasies 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 included and tested for isotopic red mass cells if appropriate.

Results: Among 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 thrombo-
cytemia, 0.04 vs 0.09, p=0.004) 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 thromb-
etic 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 thrombo-
etic events was also different with 50/50 vs 25/131. 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 thromboses and phenotypic evolutions than ET without AP. Despite these patients have a higher overall survival. So, the presence of AP in ET with characteristics patients with high risk of morbidity (thromboses, pheno-
typic evolutions).So as in PV, the presence of AP in ET patients at the time of diagnosis should be systematically identified.

ANAGRELIDE RESPONSE ACCORDING TO THE MOLECULAR PROFILE: SOMETHING TO CONCLUDE ON THE MECHANISM OF ACTION OF THE DRUG IN MYELOPROLIFERATIVE NEOPLASMS (MPN)?
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Background: Ph-negative MPNs are a heterogeneous group of stem cell derived, clonal bone marrow disorders characterised by increased production of mature blood cells. Patients with MPNs are at significantly increased risk of thrombotic and haemorrhagic complications which are a major cause of morbidity and mortal-
tality. The early diagnosis and treatment of MPN may reduce the incidence of thrombotic complications and the associated morbidity and mortality.

Aims: We performed a study to determine if the delayed-diagnosis of MPN was common and the implications of any such delay.

Methods: The medical records of patients treated at our centre with a new diagnosis of MPN between January 2010 and June 2016 were audited. We determined the duration from first appearance of a full blood count (FBC) abnor-
mality consistent with the diagnosis of MPN until the time of formal diagnosis. The occurrence of any thrombotic or haemorrhagic complications during this time was recorded.

Results: 143 patients were diagnosed with MPN: 35 with polycythemia vera, 79 with essential thrombocythemia, 25 with primary myelofibrosis and 13 with MPN-unclassifiable. Patient diagnosis median delay was 156 days (range 0-2650 days) and 26% had potentially preventable events. Patients with ET had median diagnosis delay of 823 days (range 0-8731 days) and 23% had potentially preventable thrombotic events including 2 patients with multiple events. Patients with PMF had a median diagnosis delay of 196 days (range 0-3684 days) and 12% had potentially preventable thrombotic events. In MPN-U the median diagnosis delay was 1371 days (range 42-3255) and 31% of patients had potentially preventable adverse events.

Summary/Conclusions: Over 5.5 years we identified 143 patients with a new diagnosis of Ph-negative MPN within our centre. The overall median diagnosis delay was 723 days (0-8731) with delays of more than 12 months in ET, PV and MPN-U, and more than 6 months in PMF. 21% of patients had potentially preventable thrombotic events and 2.8% had potentially preventable haemor-
ragic events. Earlier recognition of FBC abnormalities consistent with MPN may have prevented these clinical events. In the absence of an earlier diagnosis with earlier intervention, would be expected to prevent many thrombo-haemor-
ragic complications and reduce MPN-associated morbidity and mortality.

ESSENTIAL THROMBOCYTHEMIA WITH AQUAGENIC PRURITUS: AN ENTITY WITH MORE AGGRESSIVE CLINICAL AND BIOLOGICAL PROFILE AT THE DIAGNOSIS AND A HIGH MORBIDITY DURING THE FOLLOW-UP. C. Le Gall-Ianotto1,2,*, R. Le Calloch3, L.-M. Mollard4, L. Miseri1, J.-C. Ianotto4
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etic events was also different with 50/50 vs 25/131. 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).

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typic evolutions).So as in PV, the presence of AP in ET patients at the time of diagnosis should be systematically identified.
(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 two factors to be significant in predicting MF and leukemic transformation.

Methods: We aimed to find out if there is difference in frequency and type of thrombosis in JAK2V617F 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.

**Figure 1.**

Summary/Conclusions: Our experience with an alternative long-term and low-dose BU administration is safe and effective in elderly patients with ET. 92.6% of them obtained CHR, with acceptable hematological and extra-hematological toxicity. We noticed a high rate of MF evolution with respect to global population, while the risk of leukemic transformation seems to be limited,

Aims: We aimed 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.

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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.
Non-Hodgkin & Hodgkin lymphoma - Biology

E1353
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 Bcl2-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 B cell lymphoma.

E1354
CONCOMITANT DOUBLE 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)- 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-/p53- dual loss in B cells; 2) To provide an in vivo model that mimics human ABC-DLBCL phenotype.

Methods: Cre recombinase under the control of CD19 promoter (C57BL/6 CD19Cre) mice were crossed with either C57BL/6 BLIMPflox/flox or C57BL/6 Cre recombinase under the control of CD19 promoter (C57BL/6 ABC-DLBCL phenotype.

1/p53 dual loss in B cells; 2) To provide an in vivo model that mimics human ABC-DLBCL phenotype.

Results: CD19/Bl-/p53- mice presented with diffuse lymphadenomegaly, splenomegaly, hepatomegaly (100%, 90.3% and 77.4%, respectively). Other clinical manifestations included presence of ascites and hind limb paralysis (12.0% and 19.3%, respectively). The CD19+/Bl-/p53- showed improved survival 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 bimorphic cell populations, undifferentiated mostly centrally situated nuclei. A strong positive CD3 and CD20 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+/lgM+, with either Igk- or Ig-lambda-restriction as demonstrated by flow cytometry; and either mono- or bimorphic cell populations. Southern blotting demonstrated by Southern blotting. Viability assays 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 IgM+ cells. B220 positive cells were selected from the s.c. tumor and intravenous injected into recipient SCID/Bg (n: 10) and BL/6 mice (n: 10). Engraftment was demonstrated in all tumors where hepatomegaly and splenomegaly was 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.

Summary/Conclusions: Dual inactivation of p53 and BLIMP in B-cells supports the concept that what seen in patients with ABC-DLBCL, driven thus providing a novel model for studying high-grade B-cell lymphoma driven by BLIMP1-1/p53- dual loss-induced c-Myc expression.

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)(p23;q35) 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 wild-type irradiated mice.
ray analyses. Indeed, heatmap analyses revealed wide pattern similarities in the expression and number of lymphocyte subpopulation in contrast to the DN1 and DN2 lymphoma cells. Interestingly, DN3 and DN4 cells show different expression profiles of stemness genes resembling early progenitor cell distribution patterns.

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 NKFB 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 therapeutic targets in many cancers. Among the different HSPs, HSP110 has been recently identified as a pro-survival factor in germinal 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. SH-SY5Y specific for HSP110 was transduced through a lentiviral vector designed to inject highly efficiently non-permissive B cell lines.

Results: We observed a high HSP110 expression in all ABC-DLBCL patient samples, compared to normal reactive lymph nodes by using IHC staining of ABC-DLBCL patient tumors. Furthermore, RNA and protein analysis of HSP110 decreases the survival of several ABC-DLBCL cell lines, and downregulates the expression of pro-survival factors such as Bcl2 and Bcl-XL. SiRNA silencing of HSP110 abrogates NF-kB signaling, which is the major oncogenic pathway in ABC-DLBCL cell lines. In accord with these results, over-expression of HSP110 in DLBCL and non-DLBCL cell lines increases NF-kB signaling, indicating a tight interplay between HSP110 and the NF-kB pathway. Using immune-precipitation in DLBCL cell lines and Duolink™ assays, we identified an in vitro and in cellulo interaction between HSP110 and MyD88, a critical protein of the NF-kB pathway that bears an activated mutation in many ABC-DLBC patients and that is responsible for lymphoma aggressiveness. Finally, we demonstrate that HSP110 stabilizes the wild type as well as the mutated form of MyD88, therefore facilitating the chronic NF-kB pathway activation in those cells.

Summary/Conclusions: In conclusion, we identified HSP110 as a regulator of NF-kB signaling through MyD88 stabilization in ABC-DLBCL. This finding highlights HSP110 as a new potential therapeutic target in DLBCL and potentially in other hematological malignancies driven by mutated MyD88 as Waldenstrom macroglobulinemia.

E1357

STAT3 ACTIVATION MEDIATES CD8+/CD16+ /CD56- T-LGLL NEUTROPHENIA THROUGH NF-kB 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 CD8+ T-LGLL, two other LGLL subtypes have been described: CD4+ T-LGLL, less frequent LGL proliferations with CD4+/CD8-/+dim phenotype (CD4+ T-LGLL) 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 likely to be multifactorial, comprising both tumor (i.e. soluble Fas ligand 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 by 705 levels were studied by Western blot. FAS ligand mRNA levels were analysed by RT-PCR assay.

Results: By flow we observed that 68 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+ (n=14) or CD4+ (n=20). 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.

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.
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 Ccnd2 overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the Ccnd2 gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).

Results: The resulting R26-Ccnd2 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 monoclonal small-sized cells with slightly irregular hyperchromatic nuclei and disseminated into other organs such liver, spleen and the gastrointestinal tract, hallmarking the infiltrating non-malignant (N)2 lymphoma cells. These 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 assess 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 of novel proteins in the cell. However, our experiments suggest that the translation of CD20 mRNA on polysomes without affecting general synthesis of CD20 we optimized Click-IT chemistry methods. In order to study this effect of HDAC6 inhibition on global as well as specific novo synthesis of CD20 we optimized Click-IT chemistry methods. In order to study CD20 translation on polysomes we performed polysomes profiling followed with 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).

Methods: We used qRT-PCR and Dual Luciferase Assays in order to determine the influence of HDAC6 on CD20 transcription. We used pulse-chase using assays widely used with translation inhibitors – cycloheximide and homoharringtonine. In order to study the effect of HDAC6 inhibition on global as well as specific novo synthesis of CD20 we optimized Click-IT chemistry methods. In order to study CD20 translation on polysomes we performed polysomes profiling followed with 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 mRNA but rather is due to increased translation of CD20 mRNA on polysomes without affecting general synthesis of novel proteins in the cell.

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.
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, CONTINGENT OF PROLIFEROCYTIC CELLS AND TP53 ABNORMALITIES. A GFCH STUDY


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, t(7;14)(q21;q32) and t(7;17)(p11;q11), leading to juxtaposition of CDK6 gene with IGK, IGH or IGL locus respectively.

Aims: The Groupe Francophone de Cytogenetique Hematologique (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, deletions of 13q14 and 7q22/7q36 loci).

Results: Our cohort included 22M and 13F, with a median age of 71 years. The involvement of CDK6 was confirmed in all cases. A t(2;7) IGK/CDK6 was found in 33/35 patients. One case had a t(7;14) IGH/CDK6, and one had a t(7;17)(q21;q11) involving the TRAD locus. There were 23 (66%) marginal-zone lymphoma (MZL), including 22 splenic MZL (SMZL) (including the t(7;14) TRAD), and 1 bronchus MALT type, 7 (20%) non-synonymous single nucleotide variants (SNV) were observed in the constant regions of four cases and in IGKC of one additional case, but not in available RNAseq data from 6 healthy volunteers (GEUVADIS project), 10 non leg type DLBCL, and 16 follicular lymphoma were obtained from NCBI publicly available datasets and collaborators. VDJ/VJ and IGH translocations and IGKC of 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 (Dühren-von Minden, Nature 2012).

Results: RNAseq analysis demonstrated an IgM isotype in all eight and VJ-kappa rearrangements in all seven DLBCL, LT cases. IGHV3 usage was observed in 7/8 cases; 4 cases expressed the IGHV3-7 gene. DLBCL, LT BCR were strongly mutated (range: 12.5-41%). No intraclonal sequence variation was observed.

Summary/Conclusions: Our data identify a clearly stereotyped receptor in the BCR in DLBCL, LT and to test for autonomous antigen-independent signaling as described for CLL (Dühren-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 datasets and collaborators. VDJ/VJ and IGH rearrangements and IGKC of 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 (Dühren-von Minden, Nature 2012).

Results: RNAseq analysis demonstrated an IgM isotype in all eight and VJ-kappa rearrangements in all seven DLBCL, LT cases. IGHV3 usage was observed in 7/8 cases; 4 cases expressed the IGHV3-7 gene. DLBCL, LT BCR were strongly mutated (range: VDJ 3.1-22.2%; VJ 0.6-13.5%). No intraclonal sequence variation was observed.

Summary/Conclusions: Our data identify a clearly stereotyped receptor in the BCR in DLBCL, LT and to test for autonomous antigen-independent signaling as described for CLL (Dühren-von Minden, Nature 2012) and non leg-type ABC-DLBCL (Koning, AACR 2016 & ASH 2016).

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 signaling as described for CLL (Dühren-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 datasets and collaborators. VDJ/VJ and IGH rearrangements and IGKC of 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 (Dühren-von Minden, Nature 2012).

Results: RNAseq analysis demonstrated an IgM isotype in all eight and VJ-kappa rearrangements in all seven DLBCL, LT cases. IGHV3 usage was observed in 7/8 cases; 4 cases expressed the IGHV3-7 gene. DLBCL, LT BCR were strongly mutated (range: VDJ 3.1-22.2%; VJ 0.6-13.5%). No intraclonal sequence variation was observed.

Non-synonymous single nucleotide variants (SNV) were observed in the constant regions of four cases and in IGKC of one additional case, but not in available granulocyte DNA of two cases with G region mutations or in the other 32 RNAseq libraries. Constant region mutations were highly specific to DLBCL, LT as compared to other DLBCL (p=0.0018) and follicular lymphoma (p=0.0013). In contrast to ABC-DLBCL, 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 signaling of the BCR signalling cascade by tamofoxifen.

Summary/Conclusions: Our data identify a clearly stereotyped receptor in the BCR in DLBCL, LT and to test for autonomous antigen-independent signaling as described for CLL (Dühren-von Minden, Nature 2012) and non leg-type ABC-DLBCL (Koning, AACR 2016 & ASH 2016).
Background: NR4A1 (Nurr1) belongs together with NR4A2 (Nur77) and NR4A3 (Nur77-OR-1) to the Nur77 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 RT-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 preclinical 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=134), EµMyc Nr4a1 -/- (n=54) and EµMyc Nr4a1 +/- (n=59), respectively. For RT-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 preclinical stage, mice aged 4 weeks (n=4 per genotype) were sacrificed and Annexin V staining and cleaved-caspase3 assay were performed on cells isolated from the spleen and bone marrow. In vivo driver-function of Nr4a1 was induced by tumor formation from Nr4a1 heterozygous (EµMyc Nr4a1 -/+; n=8) and EµMyc Nr4a1 -/- (n=11) 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 -/+ mice 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 -/- mice 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 result in 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.

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 driver pathways in 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 sample quality is limited because of poor DNA yields 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 original genomic integrity and can be used with multiplex ligation-dependent probe amplification (MLPA) and custom designed gene-targeted deep sequencing panels. Mutations of KRAS, PIK3CA, MAP2K4 and NOTCH3 were identified. In our preliminary analysis, no evidence was found for frequent mutations of KRAS, PIK3CA, MAP2K4 and NOTCH3. Using sorted non-malignant cells as a germline control we detected somatic single nucleotide mutations and indels in all investigated genes. Mutations of STAT5, PIM1, SOCS1, KMT2D occurred in at least 18% (2/11) of cases. Individual cases contained copy number aberrations such as gain of chr2 (CREL locus), focal deletions of chr4, chr7, chr16 and chr19 affecting genes such as JAK3, CDKN2D, MAP2K3 and NOTCH3. Taken together our study demonstrates that DNA extracted from the enriched cell populations is suitable for wide-scale genetic profiling.

Summary/Conclusions: A novel rare-cell-enrichment technique is suitable for genetic cHL studies and opens the possibility for the wider use of archived
FFPE tissue, thus enabling more robust study designs to answer clinically relevant questions in the field.

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 to identify the signaling pathways driving disease. RNA-seq data were compared to publicly available RNA-seq data from different haematologic malignancies.

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 expressions that are typically found in human DLBCL such as If4, Prdm1 and p53. RNA-seq analysis revealed features shared with human DLBCL: increased reads a locus of Mef2b, Mef2a, Card11 and Cd274 (PDL1) and decreased expression of Socs-1, Cdkn1a, 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-cell mice provokes a myeloid hyperplasia which mimics 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 t(11;14)(q13;q32) 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 in 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-cell related genes (e.g. precocious T-cell development 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 to be shared by all four patients. However, aberrations in the same signaling pathways were identified across individuals. From allele frequency distributions 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 identified, whereas another patient presented a diffuse clonal pattern at diagnosis and a more discrete bicolonal 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 disease, however, the functional impact of these new genes which could be targeted by novel drugs or be used as biomarkers to predict response to treatment.

E1368

NOVEL TARGET GENES OF DEREGERGATED MIRNAS IN DLBCL ENDODGENOUS AGO2 PAR-CLIP

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Background: Aberrant expression of microRNAs (miRNAs) is a widespread phenomenon in many cancers. However, the functional impact of this deregulated gene 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 such as Ago2 to reveal the targetome. In this study, we 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-HL1) 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 and the sequencing performed on an Illumina HiScanSQ system. After deduplication and alignment, T-to-C variants (indicative of miRNA-dependent targetome) were chosen as models for the present study.

Aims: We set ourselves to adapt PAR-CLIP technique 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-HL1) 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 recently developed PAR-CLIP protocol yielded sufficient RNA for building libraries 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 binding sites) were identified, and PAR-CLIP clusters called using wavClusteR (Cornoglio, 2015).

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 7,370 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-
Background: Daratumumab (DARA) is a first-in-class human 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). DARA induced growth arrest 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) (Ovredijk MB. Mabs, 2015). In Chronic Lymphocytic Leukemia (CLL), DARA induces killing mainly via ADCC and ADCP (Matas-Céspedes A. Clin Cancer Res, 2016). Furthermore, Immunomodulatory effects (Kniejck J.B. Blood, 2016) and modulation of the enzymatic activity of CD38 (Lammers van Buuren 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 Qifkit and flow cytometry. In vivo activity was assessed in prophylactic and therapeutic set ups using SCID mice subcutaneously (sc) or intravenously (iv) injected with 1x10^7 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 standard 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 with 68% of cell death (n=6) and FL (n=4) cell lines in the presence of murine 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 and reduced organ infiltration 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 combination with current therapies, both 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.
and proliferation of several B cell malignancies. BTK is a key regulator of this pathway. In a preliminary clinical study, the safety BTK inhibitor STRO-001 showed therapeutic activity in relapsed/refractory DLBCL of the Activated B-cell phenotype (ABC-DLBC) (Walter et al Blood 127pp411-419,2016). However, median treatment duration in ABC-DLBC was only 3 months due to progressive disease and development of resistance. Two acquired resistant mutations, R665W and C481S have been reported as dominant mechanisms to BTK inhibition in CLL but resistance mechanisms in DLBCL have not been fully elucidated.

Aims: To determine resistance mechanisms in the ABC-DLBC TMD8 cell line and determine new rational combinations to take into the clinic with ONO/GS-4059. Methods: The BTK inhibitor sensitive ABC-DLBC cell line TMD8 was crossed with resistant TMD8RO and TMD8RI. STRO-001 was used for this study. TMD8RO has PLC/2 R665W whilst TMD8RI lacks both BTK C481S and PLC/2 R665W. Cell viability and apoptosis after compound treatment were assessed using Cell titer Glo assay and Annexin V/ PI staining. Western blotting showed differences in the expression of immunoreceptor were assessed by immunoblot and Flow cytometry. The mutational status of BTK and PLC/2 in TMD8 was determined by Sanger sequencing. Results: Expression of CD74 in different lymphoma subtypes was evaluated by flow cytometry, staining CD74 in >70% of cells. Although ONO/GS-4059 induced rapid reduction in ERK and AKT activation, expression 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 ONO-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 B-cell phenotype, was decreased in these cells. The combination of ONO/GS-4059 and GS-9973 restored sensitivity to ONO/GS-4059 and induced synergetic 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 inhibition treatment in ABC-DLBC. These 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 comprised of a p-azido-methyl-phenylalanine (pAMF)-containing anti-CD74 aglycosylated human IgG1 antibody (SP7219) conjugated to a non-cleavable dibenzocyclooctynylene (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 a drug-antibody ratio (DAR) of 2. Due to its limited cell permeability, the major mechanism of action is via antibody dependent cellular cytotoxicity (ADCC) and over 72 hours induced classical apoptosis in >80% of cells. Although ONO/GS-4059 showed rapid reduction in ERK and AKT activation, expression 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 ONO-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 surface immunoglobulin M (sigM) expression was increased more than three times in these cells leading to subsequent activation of SYK. The specific SYK inhibitor ONO-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 B-cell phenotype, was decreased in these cells. The combination of ONO/GS-4059 and GS-9973 restored sensitivity to ONO/GS-4059 and induced synergetic 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 inhibition treatment in ABC-DLBC. These 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.

E1374

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 neuroradiography techniques and, more recently, by the analysis of the cerebrospinal fluid (CSF). Cytomorphological examination (CM) is still 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, for the detection of malignant B 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 indeterminate (I). FCM was performed using an 8-colour panel. A sample was classified as positive (+) if a cluster of malignant cells with a clonal distribution was observed in the CSF of patients with suspected leptomeningeal dissemination. Results: In this preliminary study, the sensitivity of FCM was higher than CM and PCR for the detection of malignant B cells. Using an 8-colour panel, a sample was classified as positive (+) if a cluster of malignant cells with a clonal distribution was observed 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 indeterminate (I). FCM was performed using an 8-colour panel. A sample was classified as positive (+) if a cluster of malignant cells with a clonal distribution was observed 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 indeterminate (I). FCM was performed using an 8-colour panel. A sample was classified as positive (+) if a cluster of malignant cells with a clonal distribution was observed 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 indeterminate (I). FCM was performed using an 8-colour panel. A sample was classified as positive (+) if a cluster of malignant cells with a clonal distribution was observed 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 indeterminate (I). FCM was performed using an 8-colour panel. A sample was classified as positive (+) if a cluster of malignant cells with a clonal distribution was observed in the CSF of patients with suspected leptomeningeal dissemination.
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 569 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 optimal amplification of the BCR or venetoclax sensitive cell lines, whereas the levels of BCL2 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 optimal FCM detection). Thus, venetoclax sensitive cell lines 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 cluster 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 T-cell malignancies. It seems premature to make clinical decisions based on one technology. Both methods, which suffer limitations that need to be acknowledged, are complementary and should be performed at diagnosis. Specific limitations of each one of them should be taken in consideration for follow-up studies.

**E1375**

**THE SYK INHIBITOR R406 DRAMATICALLY INCREASES THE SENSITIVITY OF GCB AND ABC DLBCL CELL LINES TO THE BCL-2 INHIBITOR VENETOCLAX**

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**Background:** The BCL-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 BCL-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 (Davis MS et al, J Clin Oncol. 2017).

**Aims:** Investigate whether the SYK inhibitor R406 can increase sensitivity of DLBCL to venetoclax in vitro and in vivo.

**Methods:** The following cell lines were used: Ly4, Ly1, Ly7, Ly18, DHL4, Toledo and BJAB (all GCB DLBCL) and U2932, DHL2, Ly3, Ly10, HBL1 and TMD8 (all ABC DLBCL). The percentage of apoptotic cells was determined by Annexin V/propidium iodide stained FCM. Expression of BCL-2 family members was determined by immunoblotting or Q-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 (Bojczuk 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 number of apoptotic cells in six of the investigated cell lines (Ly18, DHL4, U2932, Ly10, HBL1 and TMD8). 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, DHL2 and Ly3). Among these, only Toledo expressed similar levels of BCL2 and BCL-xL. Consistent with the results of Venetoclax sensitive cell lines, we detected a 20-45% reduction in MCL1 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, DHL4, U2932 and TMD8).

**Figure 1.**

**Summary/Conclusions:** These data show that the SYK inhibitor R406 can significantly increase the sensitivity to venetoclax in the vast majority of BCL-2 positive DLBCL cell lines. The mechanisms of action require further investigation, but are likely to involve downregulation of MCL-1 and upregulation of HRK in a substantial proportion of cases.
E1377

IRF4 EXPRESSION IS ASSOCIATED WITH RESPONSE OF MANTLE CELL LYMPHOMA TO BRUTON’S TYROSINE KINASE INHIBITORS

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Background: Mantle cell lymphoma (MCL) responds poorly to conventional chemotherapy. Inhibitors of Bruton’s tyrosine kinase (BTK) 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 BTK-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 BTK response in MCL cell lines and primary cells.

Aims: To identify molecules or pathways responsible for resistance to BTK inhibitor-drugs 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 BTK sensitive REC-1 cell line was continuously treated with BTKi to generate an acquired resistance model. Cultures were treated with BTK inhibitors ibritinib or 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 receptor (pY203/205), however in IRF4 silenced cells these downstream pathways were not detected (Figure 1). In REC-1 cells, expression of IRF4 was not detected in either untreated or treated cultures, whereas expression of IRF4 was detected in BTKi resistant JVM2 cells. Moreover, knockdown of IRF4 increased BTK phosphorylation (pY223), suggesting that IRF4 downregulation may contribute to BTKi resistance.

Summary/Conclusions: Expression of IRF4 is associated with resistance to BTK inhibitor drugs in mantle cell lymphoma. IRF4 as a sensitive indicator for BTK response may facilitate the identification of patients who are not likely to respond to treatment with BTK inhibitors and serves as an alternative method for monitoring treatment response.

E1379

LIQUID BIOPSY: DECIPHERING A SIGNATURE OF CIRCULATING MICRORNAS 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 infor-

domain expression in only 49%. A substantial proportion of such failures of FCM-based clonality detection can be best explained by lost surface TR expression and the limited coverage of the Vβ antibody panel. NGS-based clonality analysis can overcome these limitations, because it detects virtually all TR Vβ-JB rearrangements. On the contrary, NGS is more sensitive and therefore enables the detection of minor subclones, which has great appeal for MRD analysis. Nevertheless, flow cytometric Vβ spectratyping is a faster, cheaper, and less labourious alternative. It has the additional advantage of detecting the actual TR Vβ chain expression and of visualizing individual T-cell subsets for quantification of Vβ cell populations.

E1384

LOSS OF TPL2 KINASE ACCELERATES MYC-INDUCED LYMPHOMAGENESIS

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Background: While MYC (8;14)(q24;q32) translocation was initially identified as a hallmark of Burkitt lymphoma, a number of other B-cell neoplasms are associated with MYC deregulation. These MYC-driven non-Hodgkin lymphomas have aggressive clinical behavior and respond poorly to treatment. However, MYC-dependent lymphomagenesis is believed to require additional oncogenic alterations, such as deregulation of genes that counteract the proapoptotic functions of MYC. TPL2 is a MAP3 kinase with an obligatory role in inflammatory signal transduction on the MEK/ERK axis but little is known 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 human healthy individuals and mouse B cells from spleens of WT (C57BL/6) and lymphomagenic 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. Differentiation 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 cytokine 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 RNA 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.

Figure 1.

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.
mation in several diseases analyzable by liquid biopsies, representing minimally invasive methods for precision diagnostics and prognosis. Blood extracellular microRNAs (miRNAs) are under investigation as novel biomarkers. While tissue miRNAs in DLBCL patients have been extensively studied, only few reports, and limited to a small subset of miRNAs, evaluated the role of circulating/serum miRNA as potential prognostic factors.

Aims: To identify and validate a serum miRNA signature with prognostic value in a cohort of newly diagnosed DLBCL patients. Methods: This is a on-going 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. Treatment response was evaluated by standard Cheson criteria. The expression profile of selected circulating miRNAs described as associated with lymphoid malignancies by us (let-7c/miR-99a1/miR-125b cluster) and by previously published studies (miR-22, miR-18a and miR-20a) was evaluated by using Taqman miRNA RT-qPCR. Serum samples were 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-99a1/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-99a1/miR-125b 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 anti-CD20 antibodies. To date, the main role of intracellular calcium and metabolism has been studied. This work aims to characterize 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 intracellular calcium is decreased. Intracellular calcium remains unchanged following treatment with Rituximab. Next, we show anti-CD20 antibody treatment causes cells to increase mitochondrial respiratory capacity to compensate for reduced basal mitochondrial function. We show that inhibition of OxPhos disables the cells from being 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.

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, chemo-immunotherapy with 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 treated with Type II 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 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.

E1381
CYCLIN D1 ONCOGENIC OVEREXPRESSION LEADS TO A GLOBAL TRANSCRIPTIONAL DOWNREGULATION IN MALIGNANT LYMPHOID CELLS
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Background: Cyclin D1 is an oncogene 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 mantle cell lymphoma. The study of cyclin D1 oncogenic 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 cytometric 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 promoters in cyclin D1-overexpression was responsible for a global transcriptional down-modulation. 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.

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's 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–Stanberg cells. Moreover, clinical behavior of cHL may be directly regulated by the cross-talk between tumor cells and infiltrating immune cells.
Aims: The aim of our study was to estimate the role of microenvironment expression of immunoregulatory molecules (PD-1 ligands, IDO) and cytokines (TGF-β, 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. 78.3% of patients achieved remission (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, TGF-β, IL-13 mRNA expression levels were analyzed in fresh pre-treatment lymph node biopsies using qRT-PCR.

Results: Expression of PD-1 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 rate of relapse, comparing to those with lower 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, TGF-β, IL-13 were evaluated in 38 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=0.008). 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) rate was 88% 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). TGF-β expression was associated with the therapy with anti-CD20 mAbs. This strategy seems to be highly promising in CLL patients, often expressing very low CD20 level and do not benefit from immunotherapy.

Summary/Conclusions: Our results clearly indicate that HDAC6 inhibition sensitizes tumor B-cells to anti-CD20 immunotherapy. Therefore, we propose HDAC6 as a potential target for immunotherapy.

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 GFP gene. 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 mice.

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.
Background: Primary gastrointestinal (GI) T-cell lymphoproliferations (T-CL) are heterogeneous entities, which diagnoses are difficult to perform. T-CL include aggressive lymphoma such as enteropathy-associated T-cell lymphoma (EATL) as well as indolent monoclonal lymphoproliferations. Refractory coeliac disease type II (RCDII) is one of the indolent T-CL that complicates coeliac disease (CD) and may evolve toward an overt EATL. The differential diagnosis of RCDII from CD and RCDI is difficult and essentially based on negative expression of sCD3 and CD8 and the presence of a clonal TCR rearrangement. Lymphocytes from RCDII are dependent for survival on IL-15, which reprograms T lymphocytes towards a cytotoxic NK phenotype.

Aims: We thus 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 only expressed on indolent T-LPD (n=0/15). The NKp46 expression was also associated with a poor prognosis in GI cell lymphoma patients (OS-5years 50.5% vs 5.4%, 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 cells as well as in SNK-6 cells. When comparing with those transfected with IL-2Rα (p<0.05), which can be fully reversed by addition of anti-IL-2Rα antibody. 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.

Figure 1. Results: Expression of IL-2Rα was significantly higher in SNK-6 cells than in NK-92 cells, at both protein and mRNA levels. Expression of IL-2Rα 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/ERK pathway were upregulated in LMP1-expressing NK-92 cells compared with the negative control. Selective inhibitors of those proteins induced significant downregulation of IL-2Rα expression in LMP1-expressing NK-92 cells as well 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-2Rα-harboring lentiviral vectors, and the cell cycle assay displayed a significant decrease in the percentage of cells in the G0/G1 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, and CDK1, 4 were involved in the regulation of cell cycle with overexpression of IL-2Rα. The IC50 values to all three chemotherapy drugs were significantly increased after overexpression of IL-2Rα, which can be fully reversed by addition of anti-IL-2Rα antibody. Summary/Conclusions: IL-2Rα expression was upregulated in NKTL by LMP1-mediated activation of MAPK/NF-κB pathway. IL-2Rα can promote NKTL cell proliferation partially through regulation of cell cycle and induce chemotherapy resistance, which can be reversed by anti-IL-2Rα antibody, indicating the potential role of IL-2Rα as a therapy target in NKTL.

E1398

LMP-1 MEDIATED UPREGULATION OF IL-2Rα 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-2Rα in NKTCL, but the role of IL-2Rα in the lymphomagenesis and chemotherapy resistance and its interactions with EBV in NKTL remains to be investigated.
Aims: This study investigated the mechanism of IL-2Rα expression in NKTCL, and explored the role of IL-2Rα in lymphomagenesis and chemotherapy resistance as well as the potential role of anti-IL-2Rα treatment in NKTL.

Methods: Expression of IL-2Rα 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-2Rα expression. Proteins in the downstream pathways 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-2Rα-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 were significantly increased after overexpression of IL-2Rα.

Results: A significant downregulation of IL-2Rα expression in LMP1-expressing NK-92 cells, at both protein and mRNA levels. Expression of IL-2Rα was remarkably 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 (~ 170 ~ 180% 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 BCWM.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.

E1390

LENALIDOMIDE (LEN) DRIVES PROGRAMMED DEATH-1 (PD1) PATHWAY
UPREGULATION IN A TUMOR MICROENVIRONMENT (TME) MODEL OF
ACTIVATED LOW-GRADE LYMPHOMA CELLS
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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-2Rα in NKTCL, but the role of IL-2Rα in the lymphomagenesis and chemotherapy resistance and its interactions with EBV in NKTL remain to be investigated.
Aims: This study investigated the mechanism of IL-2Rα expression in NKTCL, and explored the role of IL-2Rα in lymphomagenesis and chemotherapy resistance as well as the potential role of anti-IL-2Rα treatment in NKTL.

Methods: Expression of IL-2Rα 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-2Rα expression. Proteins in the downstream pathways 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-2Rα-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 were significantly increased after overexpression of IL-2Rα.

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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.
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 uM) 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 potentially be targeted to overcome both the intrinsic and LEN-induced exhaustion phenotype.

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 MTT assay and flow cytometric analysis. Expression of BCL6 and its target genes was examined with quantitative RT-PCR and western immunoblotting. The therapeutic 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 inhibitorolo. In contrast, the effect of ATO on 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 proteosomal degradation and inhibits the proliferation and growth of BCL6-dependent DLBCL.

E1393

PROTEOMIC PHOSPHOSITE ANALYSIS IDENTIFIED CRUCIAL NICU 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 extralymphatic 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 oncogenic tyrosine kinase NPM-ALK results in the constitutive phosphorylation of NIPA (Illert 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 identified pathways might help to identify new drugable 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 NIPA phosphorylation. Moreover, we performed a "proteomic-phosphosite-analysis" to identify crucial NPM-ALK specific phosphorylation sites in NIPA. Based on these results, phospho-deficient NIPA mutants were generated to investigate the functional effect of this phosphorylation: MTT proliferation- and Softagar-Assays were performed after infection of BaF3 and primary NIPA-deficient MEF cells with NPM-ALK and the respective phospho-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 partner of ALK. We were able to show a constitutive phosphorylation of NIPA by NPM-ALK does not lead to changes in the SCF(NP)-complex formation. Proteomic-Phosphosite-analyses identified 10 significantly upregulated (ratio >2; Log2Fold Change) phosphorylation sites in NIPA. NIPA phosphorylation by NPM-ALK was totally abolished. To further probe biological significance of the identified residues, phospho-deficient mutants were established and transformation assays were performed. Here we were able to show drastically impaired cell proliferation of the mutants with silenced serine/threonine residues 338, 344, 370, 381 and 387 upon NPM-ALK expression.

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-dependent T cell fusion 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 ALC.

**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 been shown different prognosis. Immunohistochemistry (IHC)-based algorithms and recently, Lymph2Cx assay, a digital test based on the expression of 20 genes, have been developed to facilitate COO assignmen. ABC-DLBCL and BL2 and Raji as Burkitt lymphoma lines) with 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 antagonist. Additionally, we generated a novel CXCR4 antagonist -named WK1- by modification of the side chain of AMD070 - a commercially available CXCR4 antagonist- on cell growth and migration of aggressive lymphoma cells in vitro.

**Methods:** 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.

**Methods:** A series of 55 patients with the diagnosis of HIV-related DLBCL (N=48), high-grade B-cell lymphoma (HGBL) with MYC and BCL2 and/or BCL6 rearrangements (N=3), or HGBL NOS (N=4) was studied. The following clinical parameters were collected from records: age, gender, ECOG, extranodal and bulky disease, HIV symptoms, Ann-Arbor stage, beta2-microglobulin, HCV and HBV serology, history of opportunistic infection and of AIDS-defining illness, onset of combination antiretroviral therapy, CD4-counts and HIV loads. IHC and FISH studies were performed on tissue microarrays. RNA was extracted from FFPE samples with RecoverAll kit (Ambion, Carlsbad, California) and digital GEP was determined with the Lymph2Cx assay (NanoString Technologies, Seattle, WA). Cohen's kappa was calculated to measure the agreement between COO given by Hans algorithm and Lymph2Cx assay.

**Table 1.**
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 a potential lymphoma biomarker has been scarcely studied.

Aims: We aimed to evaluate the usefulness of EBV load in plasma as a lymphoma biomarker in HIV-infected patients.

Methods: One hundred and fifteen patients with NHL (25% 1-7 years before lymphoma diagnosis and 75% 1-12 years before lymphoma diagnosis) and in a group of HIV-infected patients also at one year before lymphoma diagnosis (N=11) and at complete response (CR) (N=34). EBER expression was studied 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), HBV and HCV 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 HBV 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.

This work was supported by 2014 SGR225 (GRE) from CERCA Programme/Generalitat de Catalunya, and by funds from Josep Carreras International Foundation, “la Caixa” Foundation and Cegelem Spain.
LY10 and OCI-LY1) with IRF6 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 evaluated using the Kaplan-Meier method (K-M) and Cox analysis.

**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: A PROGNOSTIC TOOL FOR DLBCL**

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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/HIGH (Zytovision) double-fusion probe. InfiniumHD whole-genome genotyping assay with the HumanCytoSNP FFPE-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 for 87 cases (95.6%). The DE analysis was informative for MYC, 56 cases for Bcl6, and 65 cases for Bcl2. 7 cases (11.4%) were positive for MYC translocation, 14 (25%) for Bcl6, and only 3 (4.8%) were positive for Bcl2. No cases of MYC and bcl2 double positive DLBCL were identified. Genomic DNA from FFPE tumor tissue for SNP-A was available in 66 cases, SNP-A was informative in total SNP-A profile was performed in 63 cases, with abnormalities not corresponding to known copy number polymorphisms (89% of the cases, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) homozygous deletions, 106 (32%) gains, 41 (12%) trisomies and 16 (5%) monosomies. The most common aberrations were 1p deletion, 1q gain, 6q deletions, and MYC gains (3 deletions and 12 gains, >3 aberrations) was detected in 37/66 (56%) patients. Both DE and non-DE DLBCL groups had equal rate of aberrations per case (~5 aberr/case) and shared the most common aberrations – 1p deletion and 1q gain. In contrast, 11q deletion was more common in DE, while 6q and 17q deletions were more prevalent in the non-DE group. Non-DE DLBCL group proportion of trisomic karyotypes in non-DE group than in DE (16 vs 6 cases, respectively). Cases with MYC positive (FISH) and MYC gain (SNP-A) had the median number of two chromosomal aberrations with an exception of two MYC positive cases with complex karyotypes. These two cases shared the same 9q, 11q, 14q deletions and the monosomy of chromosome 19. Finally, of the 7 cases with normal SNP-A karyotype, BCL6 FISH-positive marker was detected in 3 patients.

**Summary/Conclusions:** SNP-A analysis highlights the genomic differences between the DE and non-DE DLBCL. Our finding of MYC positive (translocations and/or gains) association with low complexity karyotype status may suggest MYC to be an early initiating genetic event.

#### E1400

**ARQ 531, A REVERSIBLE BTK INHIBITOR, DEMONSTRATES POTENT ANTI-TUMOR ACTIVITY IN ABC-DLBC AND GCB-DLBC**

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**Aims:** To assess the biological and anti-tumor effects of ARQ 531 in in vitro and in vivo model systems.

**Methods:** Biochemical inhibition and kinase profiling were assessed using recombinant proteins. The ARQ 531 binding kinetics on BTK were determined by Surface Plasmon Resonance assay. Anti-proliferative activity of ARQ 531 was tested in a MTS-based assay against a panel of hematological malignant cell lines. Pathway inhibition assessments, in vivo efficacy and in vivo target inhibition were performed in TMD8 (ABC-DLBC) and SUDHL-4 (GCB-DLBC) cell lines and xenografts. ADME and pharmacokinetic properties of ARQ 531 were also evaluated in rats, dogs and monkeys.

**Results:** ARQ 531 potently inhibited BTK (IC50=0.85 nm) and displayed long half-life (>60 min). ARQ 531 exhibited strong anti-proliferative activity in TMD8 (GI50=0.13 µM) and SUDHL-4 (GI50=0.02 µM) cell lines, it inhibited, while potent on TMD8 cells (GI50=0.002 µM), had a GI50 of 1.1 µM in SUDHL-4, a concentration not reached in human blood, consistent with published studies. Pathway analysis in TMD8 and SUDHL-4 cells showed that ARQ 531 potently inhibited both upstream activating signals (Src kinase family) and downstream signaling pathways such as AKT and ERK. Cell cycle analysis indicated that ARQ 531 inhibited cell growth through G1 phase arrest, similar to ibrutinib. In the TMD8 xenograft mouse model, ARQ 531 strongly inhibited BTK signaling, with better efficacy than reported with ibrutinib; tumor growth reduction was >90% after 17 days of dosing, with no re-growth observed for 17 days post dose interruption. In the ibrutinib-resistant SUDHL-4 mouse xenograft model, ARQ 531 potently suppressed tumor growth (>80% inhibition) compared to the control.

**Summary/Conclusions:** ARQ 531 is a potent reversible inhibitor of BTK. Its variable kinase selectivity can be used to target constitutive BCR signaling in DLBCL primarily resistant to ibrutinib, as demonstrated by the excellent efficacy in both ABC and GCB DLBCL xenograft models. These data support the clinical investigation of ARQ 531 in patients with hematological malignancies, expected to begin in mid-2017.

#### E1401

**ROLE OF GENETIC POLYMORPHISMS ON R-CHOP EFFICACY IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: AN INTERIM ANALYSIS OF A MULTICENTER PROSPECTIVE PHARMACOGENETIC STUDY**

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**Background:** Standard chemotherapy represented by the R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) regimen is successful in about 60% of patients (pts) with diffuse large B-cell lymphoma (DLBCL). Pts who do not benefit from this treatment, due to the development of tumor drug resistance, have a very poor prognosis. Currently, knowledge on reasons of treatment related failures in DLBCL are scanty and predictive biomarker of resistance are largely unknown.

**Aims:** We hypothesized that polymorphisms of genes involved in the pharmacokinetics and pharmacodynamics of drugs included in R-CHOP regimen may play a role in predicting the outcome in DLBCL pts. Thus, we designed a multicentre prospective pharmacogenetic trial aimed at identifying gene polymorphisms potentially predictive of drug efficacy/resistance in DLBCL patient group.

**Methods:** We are reporting update data of an interim analysis on the first 80 enrolled pts.

**Results:** The study includes chemo naive DLBCL pts (Ann Arbor I-IV stages) candidate to an R-CHOP standard treatment. The Ethical Committee of each participating centre approved the pharmacogenetic protocol, and all pts signed written informed consent. In this interim analysis, the impact of single nucleotide polymorphisms (SNPs) on R-CHOP efficacy was evaluated by objective response (OR) rate, progression-free survival (PFS) and overall survival.
Liferation was observable and cells where resting in G1-phase. Cells abemaciclib had no impact on cell death or sensitization since no cell pro-

CIs:

\[ CI = 0.19 \]

\[ CI = -0.03 \]

Mino

Maver-1

/ 3.33 µM AraC). Sequential administration of abemaciclib and ibrutinib had

after 24 h resulted in synchronized S-phase entry in all sensitive cell lines (e.g.

phosphorylation of Rb on serine 795 without changes in CDK 4 and cyclin D1

31,25 nM after 24 h; as cell viability was not reduced. IC50-values of sensitive cell lines (JeKo-1, MCL cell lines (Granta 519, JeKo-1, Maver-1, Mino) and primary

(AraC) and ibrutinib. Cells were pretreated with abemaciclib and exposed to AraC or ibrutinib. We observed an almost complete and reversible G1-arrest in all sensitive cell


early 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 ibrutinib, resulting

in synergistic drug effects. In contrast, simultaneous application of Abe-

maciclib protects cells from AraC treatment whereas Abemaciclib-induced S-

phase synchronization sensitizes MCL cell lines to AraC. Further analysis needed

to explore the interaction with other targeted approaches (inhibitors of the B-

cell receptor pathway) to better understand the underlying molecular mecha-

CDK4/6-INHIBITION BY ABEMACICLIB INDUCES POTENT EARLY G1-ARREST IN MCL CELL LINES AND SHOWS SEQUENCE-SPECIFIC INTERACTIONS WITH CYTARABINE AND IBRUTINIB

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 IP, 15% of pts were in the low risk group, 58.7% in the intermediate, and 28.3% in the high risk group. We performed 468 courses of R-CHOP had been administered (mean: 5.85 courses, range:

4-46), 88.7% of pts had CR to R-CHOP whereas the remaining showed PR or

SD (7.5%) or PD (3.8%). Multivariate analysis identified FCGR2A rs1801274 as a predictor of PFS (p=0.045). Pts with HR or RR genotypes showed shorter PFS than pts with HR genotype (HR: 2.437, CI: 1.025-5.823). No statistically significant correlation was found between SNPs and OS.

Summary/Conclusions: Our preliminary data obtained in a limited number of pts, show a association between a SNP of the low affinity FCGR2A gene involved in the activity of rituximab and PFS. Further insights will derive from the completion of the present study to reach the planned numbers of cases at the end of our study.

This work was supported by a grant from the Associazione Giacomo Onlus, Castiglioncello (LI), Italy to E.M. and Cassa di Risparmio di Firenze, Firenze, Italy to S.N.
CD5 POSITIVE DIFFUSE LARGE B CELL LYMPHOMA SHOWED FREQENT MYC EXPRESSION AND AGGRESSIVE CLINICAL BEHAVIOR H.-Y. Na1, J.Y. Choe2, H.-J. Kim3, J.H. Han4, H.K. Kim5, G. Park6, H.J. Cha7, J.E. Kim8,* Aims: This study aimed to investigate clinicopathologic features of CD5+ DLBCL in Koreans. 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, IFR4/MUM1, MYC, Ki-67 and EBV in situ hybridization was performed. Florescent 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 4 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, IFR4/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 66.3 months) (p<0.05) Summary/Conclusions: The first descriptive study of CD5+ DLBCL in Korea. The incidence, clinical presentation, and pathologic features including cell of origin coincide with previous reports from western population or Japanes. 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.

Reactive florid B-lineage lymphoid proliferations in HIV infection may mimick lymphoma T. Wiggill1,1, J. Vaughan1, E. Mayne1 Aims: The aim of this study was to examine the relationship between microvesSEL density in CD30 positive diffuse large B-cell lymphomas and the possibility of a seroconversion type illness should be considered. Methods: Review of the histologic features along with immunohistochemical study for BCL1, BCL2, BCL6, CD5, CD10, CD23, IFR4/MUM1, MYC, Ki-67 and EBV in situ hybridization was performed. Florescent 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 4 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, IFR4/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 66.3 months) (p<0.05) Summary/Conclusions: This is different from the pattern noted in NHL in our centre, where CD5+ DLBCL and Burkitt’s lymphoma are very common. 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). Limited follow-up data was available, with only 8 patients documented to be attending an HIV clinic for long-term follow-up.

Summary/Conclusions: In the setting of HIV, reactive conditions may mimick lymphoma and vigilance is needed in the confirmation of monolymphoma. 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 DLBCL 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 49 (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 PFS 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: Expansions of T-large granular lymphocytes (T-LGL) with a characteristic CD3+CD8+CD57+ phenotype may be either idiopathic or develop in the context of other types of relapsed lymphomas. 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%).

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 intra-family 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 chimera and tested negative for BCR-ABL transcripts. TRBV-TRBD-TRBJ rearrangements were amplified on gDNA and subjected to paired end NGS, considering 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) marging of filtered in paired reads and (iii) quality filter of stitched sequences. Filtered in silico sequences were submitted to IMGT/HighV-QUEST, and metadata was processed by an in-house dedicated bioinformatics pipeline.

Results: Only productive TRBV-TRBD-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) pronounced skewing of the TRBV repertoire; (ii) the TRBD repertoire was dominated by the germline repertoire of more than one immunodominant clonotype; (iii) in the analysis of longitudinal samples from the same patient, persisting clonotypes albeit with fluctuating frequencies (clonal drift); and, (iv) shared (‘public’) clonotypes between father and son. In 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 oligoclonal versus monoclonal T-LGL lymphoproliferations are not sharply demarcated, but rather the transition from a polyclonal cytotoxic response to a clonal and further restriction of the T-LGL leukemia is a gradual process. Repertoire restriction, public clonotypes and clonal drift strongly indicate selection by restricted (perhaps also shared) antigens in T-LGL leukemia ontogeny and evolution.

E1408
MINIMAL RESIDUAL DISEASE (MRD) EVALUATION IN LYMPHOMAS WITHIN THE FIL (FONDAZIONE ITALIANA LINFOMI) MRD NETWORK - INTER-LABORATORY REPRODUCIBILITY ON BORDERLINE SAMPLES

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Background: In B-cell non-Hodgkin lymphomas, minimal residual disease (MRD) is a highly valuable tool for the direct assessment of the reduction of the disease burden. In 2009, the four laboratories of the Fondazione Italiana Linfomi (FIL) - FIL MRD network - started a collaborative effort to harmonize and standardize their methodologies, performing QC (Quality Control) rounds twice a year for follicular lymphoma (FL) and mantle cell lymphoma (MCL) MRD assessment.

Aims: We evaluated the molecular results of bone marrow (BM) samples analysis performed during the QC rounds, to determine how borderline samples (i.e. those with a low MRD level) challenge the inter-lab reproducibility and data interpretation.

Methods: Between February 2010 and November 2016, in the context of 14 QC rounds, the FIL MRD Network labs received 188 BM (114 FL and 74 MCL) samples; 167 were analyzed by both nested polymerase chain reaction (PCR) and real-time quantitative PCR (QR-PCR). BCL2/GHMB rearrangement was analyzed by nested PCR (Gribben, 1993) and by QR-PCR (Ladetto, 2000). Clonality assessment was performed using an IGHV multiplex consensus PCR (Van Dongen, 2003) and RQ-PCR was carried out as described (Ladetto, 2000; Donovol, 2000). All analyses were conducted and interpreted according to the “EuroMRD Consortium” guidelines (van der Velden, 2007).

Results: The sensitivity and the accuracy of each molecular assay was tested, reaching a uniform sensitivity of 10−5 and a quantitative range for MRD of at least 10−4. Ninety-three percentage of the tests carried out as described (Ladetto, 2000; Donovol, 2000). All analyses were conducted and interpreted according to the “EuroMRD Consortium” guidelines (van der Velden, 2007).

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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.

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, hypergлюбulinemia, and autoimmune manifestations. Interpretation of histological and immunohistochemical data can be difficult due to the small number of tumor cells, cells surrounded by abundant polymorphocellular infiltrate. AITL could often be 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, bone, 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 TCRG and TCRB gene rearrangements were PCR-amplified according to BIO-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.
Other Non-malignant hematopoietic disorders

E1410

USEFULNESS OF CHITOSIDROSIDASE ACTIVITY, CCL18/PARC, 7-KETOCHOLESTEROL AND GLUCOSYLPHOSPHINOLINE CONCENTRATIONS FOR SCREENING OF LYSOSOMAL STORAGE DISORDERS

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Background: Gaucher (GD), Niemann-Pick Type A/B (NPAB), 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 glucosylphosphingolipine (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 NPAB, 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 activity both in plasma and CCL18/PARC, 4/9 confirmed GD status; the rest were 1 NPAB, 1 NP-C and two carriers of NP-C. Only 3/4 (75%) with suspected NP-C and one 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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Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening clinical syndrome due to a severe hyperinflammatory response. HLH is typically characterized by a very wide spectrum of clinical findings. Central nervous system affection “CSN disease” has been frequently described at presentation of HLH, during course of disease, or as isolated CNS-HLH that could precede other systemic clinical manifestations by months to years.

Aims: To study the value of CSF soluble interleukin-2 receptor alpha subunit (sIL2Rα) assay as a marker of CNS affection in children with HLH.

Methods: In this descriptive, observational study done at Alexandria University Children’s Hospital, we analyzed the clinical data of a group of patients diagnosed as HLH. After informed consent was obtained, data was collected from patients who have undergone clinical examination, brain MRI, routine CSF analysis for evaluation of CNS-HLH, and sIL2Rα measurement in the CSF (Quantikine Human CD25/IL-2Rα Immunoassay, R&D Systems). Patients were considered as “CNS-HLH positive” when they had either neurological manifestations, abnormal findings on MRI or routine CSF analysis (elevated proteins &/or pleocytosis) and as “CNS-HLH negative” when they did not show any of these findings.

Results: We analyzed the data of 9 HLH patients; 4 females and 5 males. Their age ranged from 2 months to 13 years with a median of 5 months. Six patients had genetic diseases predisposing to HLH (Griscelli syndrome type II (GSI) & Chediak-Higashi syndrome (CHS)), and 3 other patients were diagnosed according to HLH-2004 diagnostic criteria and presumed to be of familial form (FHL). Out of the 9 patients, only 5 patients (55.6%) showed clinically evident neurological manifestations; 5 patients (55.6%) had elevated CSF proteins &/or pleocytosis, and 4 patients (44.4%) had an abnormal brain MRI. Overall 7 out of the 9 patients (77.8%) were “CNS-HLH positive” versus only 2 (22.2%) “CNS-HLH negative” according to classical criteria. Interestingly, the geometric mean of CSF sIL2Rα in CNS-HLH positive group was lower than in CNS-HLH negative group (734 vs 1952 pg/ml, p = 0.094). Moreover, CSF protein level and cell counts did not statistically correlate with CSF sIL2Rα level. Several patients showed interesting observations. Among the patients with strongly positive FHL (6/7 HLH-2004 diagnostic criteria fulfilled, NK cell activity not tested), the one with the highest observed CSF sIL2Rα level (17329 pg/ml), a 2 months old infant, was “CNS-HLH negative”, but had severe bilateral papilledema (discovered during workup for suspected autoinflammatory disorder). The second patient was a 3.5 year old infant, with history of a year of “CNS-HLH” negative findings and psychomotor regression, he had severe papilledema associated with high CSF sIL2Rα (3700 pg/ml). The third patient, an 11 months old “CNS-HLH negative” infant also had a relatively elevated CSF sIL2Rα of 220 pg/ml. On the other hand, 2 twin sisters evaluated at the age of 3 months for HLH secondary to CHS (positive family history, grey hair) had also elevated CSF sIL2Rα (600 & 800 pg/ml).

Summary/Conclusions: We hypothesize that routine CSF sIL2Rα level assay could enhance earlier & better detection of CNS-HLH in children especially in cases of non-specific, atypical, genetic forms of HLH. However, further study is needed to confirm this observation. The absence of statistically significant correlation between serum and CSF levels of sIL2Rα in our patients indicates that sIL2Rα is locally produced in the CSF secondary to cellular infiltration of the CNS and could be a valuable biological marker of disease activity. Larger prospective studies are warranted to confirm these results and determine diagnostic and prognostic value of CSF sIL2Rα levels, as well as its value for follow up of CNS disease.

E1412

GAUCHER DISEASE PATIENTS EXHIBIT A HIGH EXPRESSION OF LIPOCALINE (LCN2) AS POSSIBLE BIOMARKER OF RESIDUAL DISEASE Activity.

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Background: Gaucher Disease (GD) is characterized by a latent chronic inflammation in macrophages that may result in status characterized by pro-inflammatory cytokines, hyperferritinemia, hypergammaglobulinemia, altered calcium homeostasis and metabolic syndrome. Even patients under ERT do not fully revert this status and their risk to develop bone crisis, iron metabolism alterations, autoimmune disorders and neoplasm remain higher. This observation further supports the creation of a residual-residual or subclinical activity in type 1 Gaucher Disease patients (with undetectable levels of α-GDase) that may not be detected using classical monitoring. Therefore, despite promising results of recent studies to identify new biomarkers able to reflect the level of residual activity, there is a need to explore other possible strategies to identify residual disease activity in GD1 patients. Lipocaline (LCN2), a cytokine released by adipocytes, mononuclear cells and neutrophils with expression on endothelial cells, hepatocytes and other cells, has been involved into the monocyte 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) and 4 under miglustat therapy. This patient was part of a previous study QUELAFER and sere 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 determiniation and also a panel of cytokines (IL-10, IL-13, IL-4, IL-6, IL-7, Mip1a, Mip1b y TNFa), ferritin, hepcidin, chitotriosidase and CCL18/PARC were analyzed at study QUELAFER and sere 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 determiniation and also a panel of cytokines (IL-10, IL-13, IL-4, IL-6, IL-7, Mip1a, Mip1b y TNFa), ferritin, hepcidin, chitotriosidase and CCL18/PARC were analyzed. The patients included in both cohorts were 19-57 years old, with a median of 24 years.

Results: Comparison of LCN2 expression in GD1 patients revealed significant differences between groups. The expression of LCN2 was significantly lower in the naïve cohort (N=6) compared to patients on ERT (N=12) (p<0.001). Additionally, LCN2 expression in the naïve group showed a trend to be negatively associated with ferritin levels (p=0.098). As expected, expression of LCN2 was positively correlated with ferritin levels in the cohort of patients on ERT (p=0.014).

Summary/Conclusions: In accordance with previous reports, our results support that LCN2 expression can serve as a potential biomarker for disease activity in GD1 patients.
patients exhibit the higher values. In general 9 patients showed a reduction on LC2 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 LC2 expression were not founded. Global 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 GD1 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 unresponsive/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, renal dysfunction and/or fever were seen in 51% (n=21) and PR in 15% (n=6). Mortality rate at end of treatment was 41% (n=17) and at end of follow-up was 46% (n=19).

Summary: 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>57 (30-96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Female, %)</td>
<td>60 (60%)</td>
</tr>
<tr>
<td>Neutrophilic anemia (%)</td>
<td>98 (98%)</td>
</tr>
<tr>
<td>Renal dysfunction (%)</td>
<td>72 (72%)</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>72 (72%)</td>
</tr>
<tr>
<td>Flare episodes (median, range)</td>
<td>12.5 (0-34)</td>
</tr>
<tr>
<td>Platelet count (11 x 10^9/L)</td>
<td>27 (6-92)</td>
</tr>
<tr>
<td>VWF antigen (%)</td>
<td>20 (17)</td>
</tr>
<tr>
<td>C reactive protein (mg/dL)</td>
<td>20 (0-14)</td>
</tr>
<tr>
<td>Immunoglobulin levels (%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Renal transplantation (%)</td>
<td>6 (42%)</td>
</tr>
<tr>
<td>Major hemorrhage (%)</td>
<td>6 (42%)</td>
</tr>
<tr>
<td>Days of hospitalisation (median, range)</td>
<td>21 (5-60)</td>
</tr>
<tr>
<td>TTP (medium, range)</td>
<td>88 (20-189)</td>
</tr>
<tr>
<td>CR (%):</td>
<td>60 (17-92)</td>
</tr>
<tr>
<td>PR (%):</td>
<td>25 (6-62)</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>15 (1-34)</td>
</tr>
<tr>
<td>At completion of treatment: CH50 (U/mL)</td>
<td>45 (9-180)**</td>
</tr>
<tr>
<td>CR (%):</td>
<td>60 (17-92)</td>
</tr>
<tr>
<td>PR (%):</td>
<td>25 (6-62)</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>15 (1-34)</td>
</tr>
</tbody>
</table>

As compared to primary TTP using chi-squared for categorical data and non-parametric Mann-Whitney U test for continuous data. *p<0.1 **p<0.05.

Summary/Conclusions: Compared to primary TTP, secondary TTP had an initial poorer response to plasmapheresis. Patients with autoimmune diseases required more immunosuppressive therapy and rituximab. Although the final response and mortality rates showed a trend towards poorer prognosis in secondary TTP, it was not statistically significant. Further studies are needed to improve the treatment of TTP, both primary and secondary.

E1414

EVANS SYNDROME IN CHILDHOOD: LONG TERM SINGLE CENTER EXPERIENCE

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Background: Evans syndrome (ES) is a rare entity in childhood, usually presenting with a course that is chronic and refractory to treatment.

Aims: To report on the clinical and laboratory characteristics of pediatric patients with ES diagnosed and long followed at a single center.

Methods: Data covering a 15 year period and concerning 14 ES patients were retrospectively studied. Clinical presentation, laboratory parameters, diseased severity, therapeutic approaches, number of relapses, presence of complications, time of follow-up and final outcome were reported. Disease was considered active when Hb <7g/dl and/or PLT <30,000/mm3 and/or N <500/mm3, in partial remission (PR) when 7-11g/dl and/or PLT 30,000 – 10,000/mm3 and/or N 500 – 1,000/mm3, in short-term complete remission (SCR) when >11g/dl and PLT >100,000/mm3 and N >1,000/mm3 when still under or less than 12 months off treatment, and in long-term complete remission (LCR) when laboratory values as in SCR but free of treatment for over 12 months.

Results: Mean age at diagnosis was 5.4 years (18 months-12 years). Recent history of infection was reported in 3 (21.4%) and positive family history for another autoimmune disease in 5 (35.7%) patients. At diagnosis, 10 patients (71.4%) were Hb <11 g/dl, 7 (50%) with Leucopenia and 6 (42.8%) with Neutropenia. Only 12 patients (85.7%) had TTP pentad. Of these, 10 (71.4%) 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 (-3) were reported in 8/14 (57.1%) patients. With regards to outcome, 8/14 (57.1%) remained in LCR, 1 (7.1%) in SCR, 1 (7.1%) in PR, 3/14 (21.4%) in active disease, whereas 1 patient was lost to follow-up.

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.

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 cymopenias 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 40mg/kg PO PO/d x 10 days. Patients received Prednisone (100mg total dose, days +1, +8, +15, +22). CR was defined as an increase in hemoglobin (Hb) ≥2 g/dL, PR was defined as Hb ≥1 g/dL or an increase of ≥2 g/dL. Response was evaluated at day +28, months +6 and +12. Informed consent was obtained from all participants.

Results: Eight patients were included. Median age was 32 years (range 18-42), 6 were female. Median Hb at diagnosis was 5.8 g/dL (range 4.8-8.2 g/dL). All patients had response at day +28 (50% CR rate); median time to response was 12 days (range 3-17). During follow-up day 7/8 achieved CR (median time to CR: 30 days, range 15-103), all of which were sustained at 6 months. Median follow-up was 24 months (range 6-40). One patient presented disease-dependent and relapsed after 12 months, achieving a stable PR after re-treatment with low-dose rituximab. Furthermore, two patients had new-onset immune thrombocytopenia (IT; Fisher-Evans’ syndrome), without hemolysis 6.5 and 8 months.
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 2015. 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 NEUTROPHENIA 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 intermittent 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). PAN was considered in children suffering 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 109/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.

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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3Hematology, Hospital Universitario Son Espases, Palma de Mallorca, Spain

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. She had a known coronary syndrome treated with stent; at evaluation she referred chronic headache without other symptoms. The physical examination was normal. At that time, three possible diagnosis 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/hypotension 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 hematocrit 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 SPH-1, EPO-receptor mutation results in failure of bind of SPH-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 hematopoietic 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 hypertylgyricemia were present in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (1.77 x109/L) in our patients. Hemophagocytosis in the bone marrow in all patients. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (1.77 x109/L). Hemophagocytosis in the bone marrow in all patients. Hyperferritinemia 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 18 patients and of these, 10 had PRF1, 5 had UNC13D, and 3 had STX11 gene mutation. All patients were treated with HLH-2004 protocol. Of the 22 children who were placed in first remission. HSCT was performed in 9 patients (%32.5). The overall mortality rate was 57% (20 cases) in our series. Twenty children died opportunistic infection (n=10) or of disease progression (n=10).
Summary/Conclusions: In conclusion, FHL is a disease with high mortality rates and the only curative treatment is HSCT. Donor search for HSCT must be started and HSCT should be performed after the remission.

E1419

ABNORMAL MONOCYTE POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

Background: Chronic idiopathic neutropenia (CIN) is an acquired disorder of granulopoiesis characterized by an unexplained, prolonged reduction in the number of neutrophils and a generally benign and uncomplicated course. Neutropenia in CIN has been mainly attributed to increased apoptotic death of the granulocytic progenitor cells due to abnormal production of pro-inflammatory cytokines and pro-apoptotic mediators. Activated T-lymphocytes with a skewed oligoclonal/monoclonal profile and myelosuppressive properties have also have a major role in the pathophysiology of CIN.

Aims: Monocyte subpopulations display a prominent role in innate immunity but also mediate pro-inflammatory responses and T-cell activation. The monocyte subpopulation in CIN is not studies. 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 monocytic CD14+/CD15/DR/MF/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±496/μl and 412±130/μl, respectively (range 200-1800/μl and 200-700/μl, respectively). The proportion of classical CD14++/CD16- cells was significantly decreased in CIN patients (79.65%±7.60%) compared to the healthy individuals (87.90%±3.70%) (P=0.009). In contrast, a significant increase was observed in the proportion of CD16 positive cells in CIN patients (16.81%±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 subsets 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/DR/MF/cD33/CD11b** MDSCs was significantly increased in the patients (6.18%±3.92%) compared to the healthy controls (3.31%±1.74%) (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/DR/MF/cD33/CD11b** MDSCs 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.

E1420

DIAGNOSTIC VALUE OF CELL BOUND AND CIRCULATING ANTI-NEUTROPHIL ANTIBODY DETECTION IN PEDIATRIC NEUTROPENIA

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Background: The diagnosis of autoimmune neutropenia (nAIN) is one of the biggest challenges in clinical practice. Diagnostic criteria for nAIN are established but the evaluation of circulating anti-neutrophil antibodies (C-ANAs) still remains cumbersome and measured in few laboratories. Routinely, Hcy is used to differentiate LB12 patients because a good concordance has been reported between Hcy and MMA levels. However, in a study involving few cases, 26.5% patients with LB12 and normal Hcy showed high MMA levels.

Hcy increases in CIN and CBN patients and correlates with MMA levels. This study investigated whether the evaluation of D-GIFT improves the diagnostic accuracy of pediatric neutropenia. This can reduce the need for expensive and invasive investigations in CB patients.

Methods: I-GIFT and D-GIFT were performed by flow cytometry in 533 children including 174 (33%), 162 (30%), 81 (15%), 51 (10%) and 65 (12%) cases with pAIN, CIN, secondary autoimmune (sAaN), post-infection (PIN) and non-autoimmune (nAaN) neutropenia referred to this laboratory during 2002-2014, respectively.

Results: Using highly specific median fluorescence intensity cut-off values calculated by ROC curves, a positive D-GIFT was found in 49% of CIN patients, who showed similar clinical features as those included in the pAaN group. In 44 (27%) of 162 CIN patients I-GIFT was repeated 2-3 times in a year, resulting positive in 12 (27%) and 2 (5%) patients at the second and third screenings, respectively. Interestingly, 10 (71%) of the latter 14 patients showed a positive D-GIFT at the first serological screening.

Summary/Conclusions: D-GIFT evaluation improves the diagnostic accuracy of pediatric neutropenia. This can reduce the need for expensive and invasive investigations in CBN patients.

E1421

INAPPROPRIATE TREATMENT COULD MASK COBALAMIN DEFICIENCY: ROLE OF MELANOLACTIC ACID EVALUATION

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Background: Metabolic markers of cobalamin (Cbl) deficiency, such as methylmalonic acid (MMA) and homocysteine (Hcy) enable us to diagnose Cbl deficiency 1. They differentiate Cbl deficient patients from those with low serum cobalamin levels (LB12), but without a real Cbl deficiency. Hcy evaluation is fully automated and available in many laboratories, whereas MMA determination is cumbersome and measured in few laboratories. Routinely, Hcy is used to differentiate LB12 patients because a good concordance has been reported between Hcy and MMA levels. However, in a study involving few cases, 26.5% patients with LB12 and normal Hcy showed high MMA levels.

Aims: To evaluate the characteristics of patients with LB12 and normal Hcy and high MMA levels.

Methods: A prospective study was carried out in our University Hospital. Hcy levels were determined in LB12 (level <15pmomoll) patients with normal folate parameters for 18 consecutive months. MMA was assessed in those with normal Hcy. Serum B12, serum and red cell folate, and Hcy levels were evaluated using commercial automated methods. Hyperhomocysteinemia was defined by serum Hcy >17umomoll. Serum MMA was assessed by mass spectrophotometer and an increase in the MMA level was considered when MMA was >0.4nmol/l.

Results: A total of 237 patients with LB12 and normal Hcy were observed. In 27 (11.4%) MMA levels could not be determined. MMA levels were normal in 147 (70%). In 63 patients, MMA was increased (30%), including 25 cases (12%) with MMA levels >0.8nmomoll. In 48 out of 63 patients (76%), data on previous treatment were available. Of them, 40 (83.3%) patients had previously received inappropriate treatment (40% receiving folate) and 5 no previous Cbl treatment (10.5%). Only, 3 patients (6.25%) were treated with an adequate Cbl dosage.

Summary/Conclusions: MMA was increased in 30% of LB12 patients with normal Hcy. MMA levels were not predictive of Cbl deficiency, but 83% were erroneously treated, including 40% receiving folate. As a consequence, in most of these cases this erroneous treatment decreased Hcy levels to normal values, but cobalamin deficiency was masked and could deteriorate, especially when folate treatment was used without and adequate cobalamin replacement.

References:
E1424

EARLY LESSONS FROM WHOLE-GENOME SEQUENCING IN THE CLINICAL DIAGNOSIS OF ACCUMULATIVE RAPIDLY VENOUS THROMBOSIS AND THROMBOCYTOPENIA


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Background: Targeted re-sequencing has recently been adopted for the rapid diagnosis of anaemia patients whose disease is likely to have a genetic basis, however, currently results remain inconclusive in 30-60% of cases. Whole-genome sequencing (WGS), provides more uniform coverage than amplification-based panels and is allied to an unbiased approach offering the opportunity to explore both coding and non-coding regions. It is also possible to use WGS data to detect copy number variation without good resolution and sensitivity. Therefore WGS has the potential to offer an accurate molecular diagnosis in a proportion of unsolved anaemia cases and may therefore be a superior initial approach.

Methods: In cases where the diagnosis of suspected genetic origin. Proband were pre-screened with a targeted panel containing ~50 candidate genes, none of which had harboured likely causative variants. Analysis of WGS data involved Stampy for read alignment, Platypus for variant calling and Ingenuity Variant Analysis (Qiagen) for variant annotation and filtering, following B cell normalization, 11 months after rituximab administration. Infant no 1, 2 and 3) personal and family history, as well as laboratory screening at diagnosis, did not reveal presence of any other hematoologic, autoimmune or immunologic condition. In case number 4 AIHA followed the diagnosis of giant cell hepatitis. Hospitalization after rituximab administration ranged between 1 and 5 months, and multiple transfusions, administrations of intravenous immunoglobulin (maximum dose 65g/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 hemolytic 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 at a result of immunosuppression. However, infant no 1 developed asymptomatic progressive IgG hypogammaglobulinemia 11 months after initial exposure to rituximab, eventually requiring IVIG 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 viscosity. It is usually classified in two forms: juvenile, occurring in young ages. Currently, there is a lack of diagnostic tools to identify causative variants, of which 60% are in genes associated to slightly increased thrombotic potential. Therefore, this study aimed to undertake WGS in a set of patients in whom targeted re-sequencing had not been able to identify a molecular cause for the inherited anaemia, in an attempt to increase the diagnostic yield of the molecular analysis of such patients and provide novel candidate genes as causative of anaemia.

Methods: We performed WGS of 20 individuals (2 singletons and 6 trios) at 30x coverage where all the probands have a rare form of suspected genetic origin. Proband were pre-screened with a targeted panel containing ~50 candidate genes, none of which had harboured likely causative variants. Analysis of WGS data involved Stampy for read alignment, Platypus for variant calling and Ingenuity Variant Analysis (Qiagen) for variant annotation and filtering, followed by deleteriousness prediction and verification by functional data. Results: Known causative variants in a gene absent from the targeted panel were detected in two patients (25%), whereas candidate variants in novel genes not previously associated with anaemia were identified across the other six cases. Familial segregation and functional studies are underway to provide further evidence of causality for these novel variants, of which 60% are in genes with previous evidence of a role in erythropoiesis and 40% in genes with no known role in erythroid development.

Summary/Conclusions: These results illustrate the overlap in phenotypic abnormalities existing among these conditions and the importance of providing an accurate molecular diagnosis to enable correct diagnostic and clinical management of anaemia patients. We also demonstrate the benefit of using WGS over targeted resequencing given the difficulty of designing comprehensive gene panels and keeping them up-to-date as new candidate genes are identified.
ADAMTS13 <5% or TMA without baseline cause), 2. HUS (TMA with ADAMTS13 >5% and high creatinine levels, positive E. Coli 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 a 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 immunomodulatords. 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 eccluzimab 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 cancere 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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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 diseases, such as autoimmune diseases, immunodeficiency, autoimmune lymphoproliferative syndrome, tumors, medications or infections. They can be idiopathic or occur as a manifestation of other underlying disease. They can be idiopathic or occur as a manifestation of other underlying disease.

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 cytopenias 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 hundred and thirty-three patients with idiopathic 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 diagnosis of the patients. In 5 of the patients, first cytopenias were detected than the primary diseases were diagnosed after median 2 months (between 0 and 77 months). Only one patient firstly had 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 immunosuppressive therapies including rituximab, mycophenolate, and cladribine, cyclosporine A, azathioprine, and danazol. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primary disease. That diseases were diagnosed as systemic lupus erythematosus in 4 patients, hypogammaglobulinemia in 3 patients, and celiac disease in 1 patient. Cytopenias have countinued in 14 of the patients. One patient with CVID died.

Summary/Conclusions: Cytopenias 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 ANGIODEMIA. SINGLE CENTER

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Background: Hereditary angiodema (HA) is characterized with recurrent mucocutaneous angioedema, abdominal pain, edema of larynx and extremities. HA is a life treating, rare disease, it’s genetic inheritance known as autosomal dominant. The incidence of the disease ranges from 1/10000 to 1/15000. 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 concentrate and prophylactic use of danazol and antifibrinolytic drugs 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 follow 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, methylene tetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor (PAI) mutations were investigated in all patients. Risk factors during the 10 previous attacks of the study, five of the patients were male (50%) and five were female (50%) and their ages mean was 151.9±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 sibling and mother (10%) and no family story (10%). One patient had once per month (10%), four patients had no family story (10%). The mean serum value of C4 level in diagnosis was 4.7±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 plasmin activation which concentrate and prophylactic use of danazol and antifibrinolytic drugs 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.
E1429
FLOW CYTOMETRIC ANALYSIS OF TISSUE SAMPLES IN 42 ADULT PATIENTS WITH MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS
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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially fatal hyperinflammatory syndrome, which in its most common, secondary form, can be induced by infection, malignancy or autoimmune disease. Diagnosis of HLH is made when at least five of eight clinical and laboratory HLH-2004 criteria are met. However, diagnostic criteria were established based on studies from pediatric patients, and it is debated if they can be applied to adults. Assessment of these criteria can be subjective (microscopic identification of hemophagocytes), time-consuming or not easily available (e.g. molecular analyses, functional tests of NK-cells).

Aims: The aim of the study was to evaluate phenotypic findings from flow cytometric (FC) analyses of bone marrow (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 Cytopathology, 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 lymphopenia 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 lymphopenia 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 was diagnosed. In cases of confirmed hyperinflammation, patients with myeloid tumors 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. Monocytosis, which is a common character. However, monocytopenia 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 could aid 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.

E1430
BLEEDING IN PRIMARY IMMUNE THROMBOCYTOPENIA: WHO ARE MOST AT RISK?
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Background: Primary Immune Thrombocytopenia is a rare disorder in which patients are at risk of bleeding due to autoimmune-mediated platelet destruction. As such, treatment is varied. This study focused on describing the prevalence and types of bleeding events around the time of ITP diagnosis and after, as well as identify any factors that can potentially influence the risk of bleeding.

Methods: Data from the United Kingdom Immune Thrombocytopenia Registry were analysed for this study. The registry obtained its data from about 70 centers around the UK. Descriptive and logistic regression statistical techniques were used for this study.

Results: This analysis was based on 2365 (57.8% females) participants who are part of the Registry. The median age at diagnosis was 50 years (IQR 32, 66) and 77% of these patients were of European ethnicity. The commonest comorbid conditions was hypertension (23%). Median platelet count was 19 (IQR: 5, 53). Eighty percent had a platelet count below 30x10^9/L around ITP diagnosis. The most common bleeding events were skin-related (46.5%) and to the oral cavity (14.4%). About 70% of the cohort experienced at least one bleeding event at some point after diagnosis. After ITP diagnosis the most common bleeds were again skin-related (34.3%) and oral cavity bleeding (14.8%). Epistaxis had risen from 11.6% before diagnosis to 17.7%. Bleeding at other sites did drop. However, the prevalence of intracranial haemorrhage rose from 0.9% pre-diagnosis to 1.2% after diagnosis. Prednisolone (79%) and IVig (43%) were the most used drugs followed by rituximab (28%) among those who were treated. Romiplostim (15%) and Eltrombopag (9%) are used too but not any more than mycophenolate (18%) and azathioprine (22%). Fourteen percent of the cohort had a splenectomy at some point. Age but not gender or ethnicity were found to be associated with having a bleeding event around the diagnosis of ITP. Younger adults (18 to 30 years old) are less likely to experience a bleed than older adults (>70 years), who were most at risk. Platelet counts, expectedly, were associated with bleeding with those presenting with a platelet of <30x10^9/L were at higher risk. No comorbid illness or cotherapies were found to be associated with bleeding events.

Summary/Conclusions: The frequency of bleeding decreased for most sites but for some others a slight increase has been observed since ITP diagnosis. It is possible that bleeding events may have been recorded more accurately or observed more closely and over a longer period of time since diagnosis. However, control of bleeding was an issue after the diagnosis of ITP. Future analysis stratifying its findings by time periods would be beneficial in describing if bleeding events were better controlled over the last few years, especially after the introduction of new therapeutic agents and the publication of the internal consensus report on the diagnosis and management of primary ITP.

E1431
A MULTICENTRE, SINGLE ARM, OPEN LABEL STUDY EVALUATING THE EFFICACY AND SAFETY OF ELTROMBOPAG IN PATIENTS WITH SEVERE PERSISTENT IMMUNE THROMBOCYTOPENIC PURPURA (ITP) WITHIN SIX MONTHS OF DIAGNOSIS
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Background: Patients with acute ITP who fail or are dependent on steroids or intravenous immunoglobulin (IVig) are often committed to splenectomy or prolonged immunosuppression. Splenectomy is potentially curable but not without operative risk with many patients reluctant to undergo surgery, while the response to immunomodulation is often suboptimal with significant side effects. Although effective, to date, there is no published studies evaluating the benefit of eltrombopag among steroid dependent or resistant, non-splenectomised ITP patients diagnosed within 6 months.

Aims: To evaluate the efficacy and safety of eltrombopag in patients with severe “acute” and persistent ITP within 6 months of diagnosis.

Methods: A multicentre, single arm open label study involving 39 patients with refractory primary ITP, with platelet counts ≤30x10^9/L, despite a daily dose of prednisolone of 1mg/kg for at least 2 weeks from diagnosis OR (b) requiring prednisolone ≥10mg daily and/or recurrent doses of IVig to maintain a platelet of >30x10^9/L within 6 months of diagnosis. Prior splenectomy was not a requisite.

Platelets disorders
Patients with platelets <10x10^9/L will commence on eltrombopag 75mg daily while those with a count ≥10x10^9/L will commence on 50mg daily. A sliding scale dose is used for subjects of East Asian heritage. The dose of eltrombopag can be progressively increased by 25mg increment every 2 weeks to maximum of 150mg daily (patients of East Asian heritage should have a maximum eltrombopag dose of 100mg daily) if the platelet count remains ≤30x10^9/L or 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 >50x10^9/L) or minor response (MR; platelet ≥30x10^9/L with ≥75% 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 53 (42, 65) years, median (Q1, Q3) diagnosis follow-up time was 4.2 (2.2, 5.4) months, and median (Q1, Q3) screening platelet count was 21(13, 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 eltrombopag prior to week 12 [3 required new ITP therapy; 1 discontinuation was due to pregnancy]. At week 12, the median (Q1, Q3) platelet count was 50 (50, 100)mg daily. The median (Q1, Q3) dose of eltrombopag at week 12, zero (0, 5)mg daily. At week 12, the ORR was 64% (p<0.001; 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 12, median (Q1, Q3) eltrombopag dose was 130 (80, 200)x10^9/L. Of the 39 patients, 12 (31%) developed pulmonary embolism (PE), the median (Q1, Q3) dose of eltrombopag at week 12 was 50 (50, 100)mg daily. Of the 39 patients, 12 (31%) developed pulmonary embolism (PE), the median (Q1, Q3) dose of eltrombopag at week 12 was 50 (50, 100)mg daily. Of the 39 patients, 12 (31%) developed pulmonary embolism (PE), the median (Q1, Q3) dose of eltrombopag at week 12 was 50 (50, 100)mg daily. Of the 39 patients, 12 (31%) developed pulmonary embolism (PE), the median (Q1, Q3) dose of eltrombopag at week 12 was 50 (50, 100)mg daily. Of the 39 patients, 12 (31%) developed pulmonary embolism (PE), the median (Q1, Q3) dose of eltrombopag at week 12 was 50 (50, 100)mg daily. Of the 39 patients, 12 (31%) developed pulmonary embolism (PE), the median (Q1, Q3) dose of eltrombopag at week 12 was 50 (50, 100)mg daily.
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). Samples from 566 patients have been retrieved. As of 04/01/2009, 62% of the identified cITP patients were 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 the 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 retained 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 preponderance (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 <50×10^9/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, 28% had a history of diabetes, 9% had a history of hypertension, and 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.

EPIDEMIOLOGY OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ADULTS IN RUSSIAN FEDERATION (RESULTS OF REGISTRY OF NATIONAL HEMATOLOGIC ASSOCIATION)

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Background: ITP is an acquired autoimmune disorder characterized by isolated platelet reduction, which is considered chronic when it persists for >12 months. Evidence suggests that age may influence both the hemorrhagic manifestations of ITP and also response and adverse events (AEs) associated with some therapies. Changes in drug metabolism can contribute to increased AE rates in patients (pts) ≥65 yrs compared with younger adults. The oral thrombopoietin-receptor agonist, EPAG, is approved for the treatment of previously treated (eg corticosteroids, immunoglobulins) cITP pts, but limited data are available in pts ≥65 yrs old. The EXTEND study was a global, open-label, extension study that evaluated long-term efficacy, safety and tolerability of EPAG in adults with cITP who had participated in prior EPAG studies.

Aims: To describe the efficacy, durability of response, and safety of EPAG use in pts with cITP aged ≥65 yrs.

Methods: All pts on EXTEND started EPAG at 50mg/day, titrated to 25–75mg/day or less often as required, based on individual platelet count responses: to achieve counts in the range ≥50–200×10^9/L. Maintenance dosing con- tinued after minimization of concomitant ITP medication and optimization of EPAG dosing. Pts could remain on EPAG either for 2 yrs in countries where EPAG was commercially available, or for >2yrs until EPAG became commercially available.

Results: At baseline (BL), 50/302 (17%) on EXTEND were ≥65 yrs old. At BL, 42% were male, 58% female and 74% had platelet counts <30×10^9/L. Twenty-four pts (48%) withdrew early from the study, most commonly because of AEs (n=8, 16%), other reasons (n=7, 14%) and lack of efficacy (n=5, 10%). Median exposure duration was 2.3 yrs (range, 2 days to 7.9 yrs) and mean daily dose was 49.9 (range, 11–75) mg. Overall, 43 (86%) pts achieved platelets ≥50×10^9/L with rescue therapy; 37 (74%) achieved platelets ≥50×10^9/L for ≥50% of assessments; 26 (52%) maintained platelet counts continuously ≥50×10^9/L for 22 weeks (Fig 2). Median time maintaining platelet counts >50×10^9/L and twice BL values,
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 nosophyngitis (n=13, 26%), constipation (n=12, 24%), fatigue (n=12, 24%), diarrealgia, 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=3, 6%). A total of eight trials including 834 participants were included in this meta-analysis showed that thrombopoietin receptor agonists are safe, well-tolerated. Random-effects model was used to estimate pooled Odds Ratio (OR). Results: A total of eight trials including 834 participants were included in this meta-analysis. 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 was 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 absence 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.

E1438

SAFETY AND EFFICACY OF THROMBOPOIETIN RECEPTOR AGONISTS IN PATIENTS WITH PREVIOUSLY TREATED CHRONIC IMMUNE THROMBOCYTOPENIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: The current American Society of Hematology guideline recommends the use of thrombopoietin receptor agonists, eltrombopag or romiplostim as a rescue therapy for chronic immune thrombocytopenia (ITP). The efficacy and safety of those drugs have been tested in several clinical trials. However, the safety profile was not consistent throughout trials and is not yet well understood.

Aims: We herein conducted a meta-analysis of randomized controlled trials to compare the safety and efficacy of thrombopoietin receptor agonists: eltrombopag and romiplostim versus placebo in patients with previously treated chronic ITP. Our primary outcome was drug-related adverse events greater than CTCAE grade 3.

Methods: We performed a literature search in MEDLINE, EMBASE, Cochrane library, and the American Society of Hematology website up to September, 2015 by two independent authors according to PRISMA guideline. We included articles in English with the overall EXTEND study population (Bussel et al. Haematologica 2016;101[1]:SS17), with sustained platelet increases and reduced bleeding. Efficacy was well tolerated; AE rates were similar to that reported in the overall EXTEND study population, but an apparent increase in cataracts was observed in pts ≥65 yrs old (cataract incidence was 7% and 22% in <65 and ≥65 age groups, respectively). Further outcomes in patients ≥65 yrs old will be presented. Results should be interpreted with caution as almost half of the pts withdrew from the study. Efficacy is an effective treatment option for certain cITP pts ≥65 yrs; its use should incorporate baseline cataract screening and regular monitoring.

E1439

CHILDHOOD IMMUNE THROMBOCYTOPENIA: A NATIONWIDE COHORT STUDY ON CONDITION MANAGEMENT AND OUTCOMES

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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 more at risk of severe side-effects secondary to prolonged steroid therapies. Sirolimus has been shown to be effective in patients with ITP secondary to ALPS and in very few patients with primary disease or secondary to ALPS-like syndromes.

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 involvement of other cell lineages.

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 predominant lymphoproliferative syndrome, with or without involvement of other cell lineages.

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, CTLA4, 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 Mofelonelatomeffile (MMF) therapy. Overall, 17/23 (74%) patients achieved a response that was complete and partial in 12 (52%) and 5 patients, respectively. Prednisone was stopped in 67 (86%) cases. Children with mono-linear ITP achieved a response in 11/16 (68%) cases, with sustained platelet increases and reduced bleeding. Sirolimus was well tolerated; AE rates were similar to that reported in previous studies.
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

E1441
ASSESSMENT OF ROMIPLOSTIM SELF-ADMINISTRATION BY PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA AND CAREGIVERS FOLLOWING RECEIPT OF HOME ADMINISTRATION TRAINING (HAT) MATERIALS: A PROSPECTIVE STUDY
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Background: A HAT pack was designed as an additional risk minimization tool to support healthcare providers (HCPs) in selecting patients and training of patients/caregivers to mitigate medication error risk when self-administering romiplostim subcutaneously, a thrombopoietin receptor-agonist which is approved in the European Union (EU) to treat chronic immune thrombocyto- penic purpura (ITP) refractory to other treatments.

Aims: To estimate the proportion of adult patients and caregivers who admin- istered romiplostim correctly after HAT pack training.

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.

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 nurs- ing 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 admin- istered romiplostim correctly at a voluntary subsequent visit.

Summary/Conclusions: Given that this study was conducted on a conven- ience 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 adminis- tering romiplostim after HAT pack training suggests that self-administration of romiplostim is a feasible option for suitable romiplostim-treated ITP patients.

E1443
SHORT- AND LONG-TERM RESULTS OF FIRST LINE THERAPY WITH PULSED HIGH-DOSE DEXAMETHASONE IN ADULT IMMUNE THROMBOCYTOPENIA PATIENTS: A RETROSPECTIVE SINGLE-CENTER REPORT
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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder mediated by clearance of antibody-opsonized platelets (plt) by spleen macrophages. Pulsed high-dose dexamethasone (HD-DXM) has proved to be effective in adult patients (pts) with primary ITP resulting in controlled studies in 89% short-term response and a relapse-free survival (RFS) of 58% at 50 months (mos) (Mazzucconi, Blood 2007).

Aims: To assess the short-term and sustained response rates of adult ITP pts receiving pulsed HD-DXM in everyday clinical practice.

Methods: Charts of pts with ITP - as defined by Rodeghiero, Blood 2009 - treated with HD-DXM were reviewed. DXM was administered according to the schedule of 40mg/day for 4 consecutive days to be repeated every 21 days for a maximum of 6 courses. A reduced-dose schedule of 20mg/day for 4 days was preferred for elderly/diabetic pts. Pts who had completed at least 3 courses were included in the analysis. Response to HD-DXM was classified according to IWP definitions (Provan, Blood 2010); therefore, steroid-dependent pts were considered as non-responders even if plt counts increased to safe levels during HD-DXM and were included only in the analysis of short-term response, but not evaluated for long-term response. Short-term response rate was determined at completion of the whole course of treatment. Relapse was defined as a plt count decrease ≤20x10^9/L after initial response achievement and RFS was defined as the time interval between last course administered and the date of relapse, censoring pts alive or dead without relapse. Follow-up was defined as the time between diagnosis and last available assessment. The probability of RFS was calculated using the Kaplan-Meier method.

Results: A total of 45 pts (M: 21) were eligible for analysis; median age at treat- ment was 60 yrs (range 18-87) and median time between diagnosis and treat- ment start was 3 days (range 0-4686). Pts received a median of 5,15 courses (range 3-6); 27/45 completed 6 courses; 21/45 received the full dose of 40mg/day (=960mg total dose) while 6/45 received the reduced dose of 20mg/day (=480mg total dose). Median total DXM dose was 800mg/dVIO along with 1st DXM course were required in 11/45 pts. In between courses, no bleed- ing complications were observed and no emergency therapies were required. Short-term response was achieved in 38/45 (87%); complete response (CR) in 28/45 (62%), response (R) in 7/45 (16%); 4/45 (9%) pts were classified as steroid-dependent ITP and excluded from subsequent analysis. Long-term response off therapy, lasting for a median time of 28 mos (range 5-80) without relapses was observed in 25/35 responding pts (71.5%; CR in 18/25, R in 7/25 at last follow-up) with a RFS of 51% at 50 mos (Fig. 1). Median plt count at last
follow-up was 102x10^9/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=0.67), 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 daily 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, or platelet count was not available before treatment (n=38). The direct immunofluorescent antigen test was carried out with nasopharyngeal 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±9x10^9/L vs 190±13x10^9/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).

Table 1. Platelet counts (x10^9/L) in patients with a clinical suspicion of influenza and after oseltamivir treatment (median of 5 days). Results are given as mean±SD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Platelet count x10^9/L</th>
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<tr>
<td>Start</td>
<td>170±9</td>
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<tr>
<td>End</td>
<td>190±13</td>
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<td>p</td>
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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 is not ruled out, the lack of long-term fluctuations after the end of treatment may indicate a late inhibition that 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 with recognized TTP and may be a risk factor for new onset 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: TTP patients underwent cardiac MRI scanning between November 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 13-75), 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 <14ng/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 myocardial infarction. 33% of patients did not show evidence of cardiac involvement on transthoracic echocardiogram (2). A recent study confirms the effect of oseltamivir on platelet counts. MEFV gene mutations are responsible for Familial Mediterranean Fever (FMF) a hereditary autoinflammatory disease characterized by recurrent febrile inflammatory attacks of serosal and synovial membranes. MEFV gene’s product, pyrin or marenostin, play an essential role in the regulation of inflammatory reactions. MEFV gene mutations are associated with a wide range of auto-inflammatory and autoimmune diseases. Recently, studies showed that MEFV gene mutations change the Th1, Th2 balance and increase the Th17 numbers. Th17 cells may have a key role in neutrophil activation and autoimmune diseases. ITP is an autoimmune-mediated condition that results from antibody-mediated destruction of platelets and impaired megakaryocyte platelet production. ITP is a complex disorder of immune dysregulation caused by anti-ADAMTS13 antibodies produced by B cells. A recently proposed model for ITP involves antigen-presenting cells, B cells and T cells. Increased Th1/Th2 ratio and Th17 levels are implicated in the pathogenesis of ITP, as well.

Aims: This study addressed the prevalence of MEFV mutations and their effect on clinical features of ITP.

Methods: We studied the prevalence of exon 2 and 10 mutations (E148Q in exon 2, M694V, M694I, M680I, V726A, A744S and R761H in exon 10) in 81 adult ITP patients and 186 healthy controls. Patients were classified in two subgroups according to the presence of mutations. Demographic and clinical features were compared between groups to assess possible impacts of these mutations on clinical severity.

Results: Female to male ratio was 61/20=3.05 in the study group and 98/88=1.1 in the control group. The median age was 50 (21-79) in the ITP
Background: Splenectomy may lead to a good response in 60–80% of adults with corticosteroid refractory immune thrombocytopenia (ITP). However, in the era of new drugs the proper selection of patients for splenectomy is essential in optimizing treatment outcomes. Accordingly, it is important to identify pre- and post-operative parameters that are able to predict the response to splenectomy.

Aims: To identify the pre- and postoperative parameters predictive of successful splenectomy in ITP.

Methods: We retrospectively analyzed 130 ITP patients (median age 43 years, range 19–74; 84/39 female/male; median time from diagnosis to splenectomy 19 months, range 2–132; median number of pre-splenectomy therapies 2, range 0–18). The patients were divided into responder (CR and PR) and non-responder groups. The responders were defined as patients whose platelet count (PC) remained >50×10^9/L after splenectomy.

Summary/Conclusions: A higher PC on the surgery day (90×10^9/L vs.37×10^9/L, ρ=0.353, p<0.0001), a PC >250×10^9/L on the day of surgery (p=0.043) and PC >300×10^9/L on the 7th days after splenectomy (387×259/L vs.25×10^9/L, p=0.022, 0.001) and splenic platelet destruction (86% vs. 0%, p=0.035, p<0.0001). Using ROC analysis, cut-off prognostic values of PC were reevaluated: PC before splenectomy >47×10^9/L (AUC 0.864, sensitivity 63.6%, specificity 83.2%, 95% CI 0.785–0.943, p<0.0001). PC on the first day after splenectomy >50×10^9/L (AUC 0.956, sensitivity 44.4%, specificity 83.2%, 95% CI 0.912–0.999, p<0.0001) and on the 7th days after splenectomy >300×10^9/L (AUC 0.951, specificity 91.7%, sensitivity 45.6%, 95% CI 0.887–1.000, p<0.0001) were found as important predictors of the postoperative response rate.

Results: CR and PR were achieved in 105/130 (79%) and 12/130 (7.5%) of the splenectomised patients, respectively. However, 13/130 (11.5%) patients were refractory. Twenty-nine of the 117 (24.8%) responsive patients relapsed. Predictors of good response after splenectomy identified by univariate analysis were: initial response to steroids (89.5% vs. 22.7%, p=0.038, 0.001), higher PC on the surgery day (90×10^9/L vs.37×10^9/L, p=0.353, p<0.0001), on the first (387×259/L vs 56×10^9/L, p<0.0001) and on the 7th days after splenectomy (387×259/L vs.25×10^9/L, p=0.022, 0.001) and splenic platelet destruction (86% vs. 0%, p=0.035, p<0.0001). Using ROC analysis, cut-off prognostic values of PC were reevaluated: PC before splenectomy >47×10^9/L (AUC 0.864, sensitivity 63.6%, specificity 83.2%, 95% CI 0.785–0.943, p<0.0001). PC on the first day after splenectomy >50×10^9/L (AUC 0.956, sensitivity 44.4%, specificity 83.2%, 95% CI 0.912–0.999, p<0.0001) and on the 7th days after splenectomy >300×10^9/L (AUC 0.951, sensitivity 91.7%, sensitivity 45.6%, 95% CI 0.887–1.000, p<0.0001) were found as important predictors of the postoperative response rate.

Summary/Conclusions: Splenectomy is effective in approximately two thirds of patients with ITP. Our study suggests that splenectomy might be considered in the patients younger than 60 years, with splenic platelet destruction and PC >25×10^9/L on the splenectomy day.
drug reactions (ADRs), and other clinically relevant parameters. We report results of a full data analysis.

**Results:** A total of 59 patients were enrolled (49.4% male; 54% aged 65 years or above) from 38 sites; 22 of them were excluded due to protocol violations (e.g., incomplete documentation, inclusion criteria not met). Of the 137 remaining patients (the full analysis set, FAS), 102 completed the 2-year observation period. Drop-out included loss to follow-up (10 patients), deaths (6 patients) and ADRs (3 patients). Median (Q1, Q3) time from ITP diagnosis to romiplostim initiation was 21.7 months (4-5 months) in the FAS. 123 FAS patients received prior ITP therapies; most of them received corticosteroids (104 [75.9%]). 117 patients (85.4%) were non-remissioned before romiplostim therapy, for reasons such as refusal of splenectomy, comorbidities, or age. Over the observation period, romiplostim was injected at a median (Q1, Q3) dose of 3.13 mcg/kg/bw (1.8 - 4.8; FAS) over a median (Q1-Q3) treatment period of 103 weeks (33-104). The median platelet count rose sharply from baseline (29.0 x 10^9/L) to two weeks of treatment (62.5 x 10^9/L). From week 3 to baseline, the median count was maintained in a range of between 50 x 10^9/L and 145.5 x 10^9/L. Since the start of the romiplostim therapy, 59 patients out of 137 (43.1%) received concomitant therapies, mostly corticosteroids (49 patients [35.8%]). The overall number of ADRs was 112 in the FAS, affecting 37 patients (27.0%). The most frequent ADRs were gastrointestinal (10.2%) and neurological (11.7%) ADRs, followed by constitutional symptoms (10.8%). 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 the 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-remissioned. The product was well tolerated and achieved a decrease in the rate of ITP-related hospitalization.

**E1451**

**FIVE NEW CASES OF HERMSKARY-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, oclocutaneous albinism and sometimes 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 (exons 1-67 inclusives).

**Methods:** We studied 5 patients from 3 families (2 Spanish, 1 Turkish) presenting with oclocutaneous albinism. Clinical records were reviewed and bleeding scored with ISTH-BAT. Platelet phenotype (only Spanish patients) included: platelet aggregation, GP's expression and granule secretion. 14C-serotonin uptake and whole mount electron microscopy. Patients DNAs were amplified 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 Cron’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 homozygous HPS4 variant, c.272T>C (p.Leu91Pro), was found in two Turkish siblings (F2). One had severe GI bleeding requiring cologney (P4) 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.

**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 enhanced 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), using flow cytometry and correlation between sGPVI and PF4 levels.

Methods: 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 (598×10^9/L versus 297×10^9/L; p=0.04). Similarly, patients with HELLP syndrome demonstrated increased levels of sGPVI than those without HELLP; corrected for platelet count (2.576±0.9667 µg/ml versus 1.22±0.124 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 3.71±0.74 µg/ml compared with non-severe patients. 1.85±0.35 µg/ml (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 previously published trials 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 the same indication.

E1454 PRIMARY ITP IN ADULTS TREATED WITH ELMOTRUBAG: 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. First-line treatment has remained unchanged for decades and until recently, second-line therapy has been unsatisfactory, using empirical treatments. The recently approved thrombopoietin receptor agonist eltrombopag and romiplostim have transformed patient care and these agents are licensed second-line therapies in adults.

Aims: To describe the adult patients receiving eltrombopag using data from the UK Adult ITP Registry. In particular we were interested in understanding the mean dose used, number of prior therapies, median length of treatment with eltrombopag, median counts at baseline before treatment and at six months following treatment, and sustained response in patients who have received eltrombopag.

Methods: The UK Adult ITP Registry involved more than 70 UK collaborating centres, coordinated by The Royal London Hospital. In this study we analysed data from all patients receiving eltrombopag and analysed these using various statistical techniques.

Results: The total number of patients evaluated was 129. The median age at diagnosis was 49.4 years (26.9-66.4). There were 74 males (57.4%) and 55 females (42.6%). 29 patients (22.4%) had undergone prior splenectomy. The median age at eltrombopag initiation was 59.5 years (37.0-70.7 years). The median time from ITP diagnosis to eltrombopag initiation was 1.6 years (0.7-2.3 years). The majority of patients started eltrombopag between 2013 and 2016 (93%), and 6% (9) started eltrombopag within the first 6 months and between 6 to 12 months of ITP diagnosis, respectively. Most patients had received prior ITP therapies. Some 10 patients (7.8%) had received one prior ITP therapy and 99 patients (77%) had received three or more prior therapies before starting eltrombopag. The commonest prior therapies were corticosteroids in 110 patients (87%); IVg 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 21×10^9/L (10-54) and the majority of patients (64.5%) had platelets less than 30×10^9/L. The mean platelet count at 6 months was 206.2×10^9/L and at 1 year was 288×10^9/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%), IVg (33%), corticosteroids (15%), and rituximab (14%) were added. 22% of patients (22%) 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 100x10^9/L and 27 (25%) had a partial response (platelet counts between 30 to 100x10^9/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 adults ITP. Only 10 patients (7.8%) had received romiplostim as a 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: 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 26 to ELMOTRUBAG. Median age was 69 years (range 39-94 years). In the group of patients treated with Romiplostim, 21 had already received 3 or more prior therapies, whereas 18 had received only one prior treatment. 13/26 patients who received ELMOTRUBAG were at the 3rd line of therapy. At the second and, the others were at the 4th line. The median platelet count was 21x10^9/L (3-52) at the start of Romiplostim, with a median starting dose of 1 µg (1-2) and 17x10^9/L (1-53) in patients treated with ELMOTRUBAG, with a median starting dose of 50mg (25-50).

Results: Patients treated with Romiplostim we observed 22 complete responses and 10 responses, with a 82% response rate, while 7 patients were no responders. In our study 26 (66%) patients stopped Romiplostim after a median time of 16 months (1-93): 9 for stable response, 5 for no response, 3 for loss of response, 3 for adverse events (2 for bone marrow fibrosis, 1 for headache and myelodysplastic syndrome); we observed 3 patients stopped the line of therapy, 5 patients stopped the line of therapy, 2 underwent a splenectomy, and 3 patients interrupted the treatment for other causes (es. diagnosis of cancer). The median platelet count at suspension of Romiplostim was 91.5 x 10^9/L (3-320). In patients treated with ELMOTRUBAG 16 achieved a complete response, 5 a response, obtaining response in the 80% of cases; 5 were no responders. 14 (53%) patients stopped ELMOTRUBAG after a median time of 15 months (1-12): 6 for adverse events (2 cases of major cardiovascular events, liver toxicity, skin rash, pharyngitis), 5 for no response, 1 for loss of response, 2 patients who achieved a CR interrupted ELMOTRUBAG obtaining a sustained remission after discontinuation. The median platelet count at suspension of ELMOTRUBAG was 57x10^9/L (7). In patients treated with ELMOTRUBAG we did not interrupt treatment. The patients were still receiving therapy with a median of 29 months (3-96). Several studies reported Romiplostim and ELMOTRUBAG to be highly effective against chronic ITP, with average immediate responses exceeding 80% in our study. We observed that therapeutic response was influenced by the starting platelet count. In particular patients count before therapy influenced the first response observed. In particular in patients treated with Romiplostim PLT pre-treatment directly correlated with the first response and the maintainance of response during treatment at month 1°, 2° and 3°. Patients with a median starting platelet count of 15x10^9/L obtained a response (CR + R), while almost all patients who started therapy with PLT<15x10^9/L at baseline can obtain an initial response, but the majority is not achieved. If the response is not achieved on the first 3-5 weeks, the treatment should be interrupted.

Summary/Conclusions: TPO-mimetics have proved efficacy in patient with ITP and their use can be applied in several conditions (bridge to splenectomy; sustained response; switch and discontinuation). Future study on large series of patients are needed to best correlate baseline platelets with hematological response. Furthermore, the two current TPO RAs (Romiplostim and ELMOTRUBAG) 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.
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 Roddighero 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 of ITP (P=0.015), longer interval since ITP diagnosis (P=0.001), persistent or chronic ITP (versus acute, 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 (RR, 3.830; 95% CI, 1.111-13.196; P=0.033), and smoking (RR, 2.622; 95% CI, 1.250-5.499; P=0.011) were independent risk factors for TEE. When the variable “number of treatments” (<2 versus ≥2) was excluded from the multivariate model, having a splenectomy increased the risk for TEE. The cumulative incidence of TEE at year 1, 5, 10, 15 and 20 years since diagnosis of ITP was 6.2% (95% CI, 4.1-9.3), 11.9% (95% CI, 8.3-17.0), 15.8% (95% CI, 11.1-22.4), 24.2% (95% CI, 16.9-34.7) and 32.8% (95% CI, 22.8-47.3) respectively (Figure). Death occurred in 7/44 (16%) patients with TEE, and in 12/394 (3%) patients without TEE (P<0.0001). Most frequent causes of death were infection (32%) and bleeding (21%).

Methods: We performed a prospective study in 4 ITP patients who exhibited no response to standard therapies (steroid, IVIG and/or splenectomy) and showing relevant platelet desialylation levels. Patients were given off-label oseltamivir at the referring physician’s discretion. Desialylation of GP platelet surface was examined via flow cytometry (FC) analysis, with fluorescence-conjugated Rcinus Communis Agglutinin I (RCA-1), which binds galactose residues only if the terminal sialic acid has been removed. FC data are expressed as fold change compared to control samples. Additionally, patients’ sera were incubated with normal human platelets to analyze the ability to induce desialylation of normal platelets. Analysis of plasma proteins was performed by Western blot (FXI, FXIIl and HPLC [carbohydrate]. Platelet autoantibodies specificity was detected by a solid-phase modified antigen capture ELISA test (MACE).

Results: Patients’ characteristics are summarized in Table 1. Two patients achieved complete platelet response (>100x10^9/L after oseltamivir treatment. The oral dose was secured eight times daily, for a variable duration (5 days in one case and 4 months in the other showing response criteria since the third week of from start) combined with low doses of other treatments (azathioprine or romiplostim). A sustained platelet response was observed after 4 weeks of the sial-
idase inhibitor discontinuation. Patients with no response after oseltamivir treatment (n=2) were given similar doses for 5 days. Patients with response had antibodies directed solely to GPIb and had greater platelet loss of sialic acids. Moreover, their sera induced significant desialylation of normal platelets. However, no desialylation in patients' plasma proteins was detected. Biological analysis after treatment discontinuation (median of 3 weeks), revealed a sustained sialylation level of platelet glycoproteins over time, particularly in patients with sustained platelet response.

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
BORTEZOMIB 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 amounts 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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haematologica | 2017; 102(s2) | 595
Figure 1.

Summary/Conclusions: Administration of oral analgesia and anxiety 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 anesthesia alone or sedo-analgesia plus local anesthesia.

Table 1.
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 + eltrombopag 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 IST. 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 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 ($96,816) 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 (approx. €11,781) and SEK 135,655/quality-adjusted life-year (approx. €14,922), 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 anaemia.
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 armamentarium 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 on or after 1st January 2014, to specifically examine the HCRU post 1L ASCT. Data collected pertained to pt characteristics, b-parameters, 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%, 26% 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 8% 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 (12%). The TTP from start of 1L ASCT 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 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 tx); mean visits to a HCP during this period were 17. The mean TTP from start of 2L tx was 11.2 months (±6.2 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 clinical trials. 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.

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 chemotherapy regimens for the treatment of children, adolescents, and adults with acute lymphoblastic leukemia (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.

Methods: In line with accepted National Institute for Clinical Excellence (NICE) methodology, a combined decision tree and health state transition Markov model was developed to compare treatment sequences starting with PEG-ASP versus native ASP, followed by ERW-ASP in case of hypersensitivity. Although ERW-ASP is not used first-line in the United Kingdom, alternative switching scenarios could be clinically possible, and therefore all scenarios were modelled. Paediatric, young adult (≤25 years), and adult (≥26-65 years) patients were modelled separately using the UKALL 2003 and UKALL14 protocols, respectively. Further splits were made between high-, intermediate-, and standard-risk patients in the paediatric model, between patients aged ≤40 vs >41 years and patients eligible vs not eligible for transplant in the adult model. Key model parameters (survival, risk of hypersensitivity) were based on published data and clinical expert input. In the base-case analysis, overall survival and event-free survival were assumed to be equivalent for PEG-ASP, native ASP, and ERW-ASP, with 1,000,000m22 dosage (per UKALL protocols) used for both. The 2,000 IU/m2 dosing of native ASP was examined, as well as variations in comparative survival and hypersensitivity rates. Incremental cost-effectiveness ratios (ICER; defined as incremental costs/quality-adjusted life years [QALYs] gained) were produced.
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.

E1466 IMPACT OF VENETOCALAX ON THE QUALITY OF LIFE OF PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA: RESULTS OF A PHASE 2, OPEN-LABEL STUDY OF VENETOCALAX (ABB-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 sustained an 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 QLG-C30 and EORTC QLG-CLL16, which were assessed at Baseline (BL), at 4 weeks and every 12 weeks thereafter. Mean change in the HRQoL measures from BL to each assessment are reported. Clinical relevance was based on minimum important difference (MID) of values from BL at different assessment points. The lower bound of 5–10 point changes, considered a “little” change for EORTC-C30 global health status and the role, social, and emotional functioning scales. Improvements in VEN treated patients in EORTC-QLQ-C30 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-QLG-C30 and EORTC-QLG-CLL16 (Table 1). The changes observed in patient EORTC-QLG-CLL16 future health views were considered large (>20 points) at Weeks 12, 24, and 48.

Table 1.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>10 points and percentage correct answer</th>
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<tr>
<td>Summary/Conclusions: These updated interim results suggest that patients receiving VEN monotherapy experienced early and sustained clinically relevant improvement in several key aspects of functioning and HRQoL for up to 96 weeks in a very symptomatic and difficult to treat patient population. These results are important to consider when making treatment decisions in the R/R settings.</td>
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E1467 WHICH HAEMATOLOGICAL CONDITIONS CAN THIRD YEAR MEDICAL STUDENTS RECOGNISE INTERPRETING FULL BLOOD COUNT RESULTS?

S. Lovato1,2,*, J. Arnold1,2

Summary/Conclusions: These 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 in a different group during the t-RAT and worked as peer-to-peer teachers for the other students. The i-RAT results for AML were actually worse than for the i-RAT, probably the students who replied correctly in the i-RAT were concentrated in fewer groups. To the authors’ 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.
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 disease.

Aims: This prospective, randomized and controlled study tested the safety, feasibility and efficacy 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. Target 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 EORTC QLQ-C30, the Short Physical Performance Battery and the 6 Minute Walking Distance between both groups was 23 meter (p=0.2). SPPB test results differed statistically (p<0.05) or ventricular arrhythmias occurred. Difference in 6-minute walking distance between groups was 23 meter (p=0.2). SPPB test results differed statistically (p<0.05) or ventricular arrhythmias occurred. Difference in 6-minute walking distance between both groups was 23 meter (p=0.2). SPPB test results differed statistically (p<0.05) or ventricular arrhythmias occurred. Difference in 6-minute walking distance between both groups was 23 meter (p=0.2).

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 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 physiological and mental component summary scores (PCS and MCS). HCU (e.g., outpatient visits, hospitalisations, costs) and the duration of disease. There were significant associations between PCS and ER visits (p<0.05) and between both types of EMS stimulation 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.

Summary/Conclusions: Findings from this study suggest that not all myeloma patients value the same treatment features equally. This finding has important implications for the healthcare policy decision to be supported by the HCU decisions of patients living with others and those diagnosed in the last five years were more likely to be in class one (those more concerned with overall survival) than class two (those more concerned with quality of life).

Background: There is a lack of real-world evidence regarding HCU associations between HRQoL and HCU, indicating there is potential for using HRQoL surveys as screening tools to predict future HCU for 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. The development of prediction models for HCU in AL amyloidosis should consider incorporating HRQoL, as well as disease staging and treatment type.

Aims: The study aimed to answer the following questions: What treatment attributes do myeloma patients value? What is the relative importance of different preferences of each patient group? Therefore, myeloma patients face difficult decisions about the benefits and risks of treatment. The purpose of this study was to assess myeloma patients’ preferences for treatment.

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 associated benefits of the treatment.

Summary/Conclusions: Findings from this study suggest that not all myeloma patients value the same treatment features equally. This finding has important implications for the healthcare policy decision to be supported by the HCU decisions of patients living with others and those diagnosed in the last five years were more likely to be in class one (those more concerned with overall survival) than class two (those more concerned with quality of life).
E1472
QUALITY OF LIFE AND ABILITY TO WORK OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH THYROSINE KINASE INHIBITORS
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2Hematology department, University hospital of Tiemcen, Tiemcen,
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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 these areas 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.

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 20-60), 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% worked 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 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 3984 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) anthracyline treatments. Impact of anthracycline on diabetes was further studied by multivariate Cox proportional hazard regressions in a dose-dependent manner.

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, anthracyline 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 anthracyline 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 anthracyline treatment had similar yearly adapted diabetes complications severity index alteration (0.58±1.89 vs 0.75±1.85; mean±standard deviation), suggesting anthracyline did not deteriorate outcome of diabetes in B cell lymphoma patients (p=0.4924).

Table 1.

Table 2. Levels of quality of life reported

<table>
<thead>
<tr>
<th>Modality</th>
<th>Median score (range)</th>
<th>Percentage of participants</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global QL</td>
<td>80 (60-100)</td>
<td>71%</td>
<td>80±10</td>
</tr>
<tr>
<td>Physical QL</td>
<td>80 (60-100)</td>
<td>83%</td>
<td>80±10</td>
</tr>
<tr>
<td>Role QL</td>
<td>80 (60-100)</td>
<td>83%</td>
<td>80±10</td>
</tr>
<tr>
<td>Cognitive QL</td>
<td>80 (60-100)</td>
<td>83%</td>
<td>80±10</td>
</tr>
<tr>
<td>Social QL</td>
<td>80 (60-100)</td>
<td>83%</td>
<td>80±10</td>
</tr>
<tr>
<td>Emotional QL</td>
<td>80 (60-100)</td>
<td>83%</td>
<td>80±10</td>
</tr>
</tbody>
</table>

Figure 1.
Summary/Conclusions: Anthracycline therapy was responsible for more deaths by 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 LENALDIAZOM 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 (mMM) 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 local 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 mMM 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 mMM 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 regimes. 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 mMM patients with health utility adjustment. The model took into account (i) drug acquisition costs, (ii) treatment administration costs at the Chinese urban setting, (iii) serious adverse events management costs based on a survey of seven MM centers across China, and (iv) mMM management costs estimated from a Chinese real-world hospital setting. Quality-adjusted life years (QALY) and direct medical costs in the model were discounted at 3% per annum. Base case analysis calculated incremental cost effectiveness ratios (ICERs) per QALY for RD relative to 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-analyzing the published meta-analysis of randomized controlled trials for the efficacy associated with RD, VD, and VCD, to verify the base case analysis.

Results: Based on the model simulation without discounting survival outcomes over a lifetime horizon, RD could obtain longer average lifetime PFS years and QALY with (2.37 vs 0.78) and VCD (2.37 vs 1.36). RD was associated with longer duration of response (14.11 vs 14.11) and lower treatment costs ($494,060 vs $272,135 and $244,220) than both VD and VCD. The ICERs per QALY for RD relative to VD ($149,706) and VCD ($150,774) were less than the cost-effectiveness threshold of China (three times of estimated 2016 China GDP per capita $166,920/QALY, $1= €0.138). The cost-effectiveness of RD was robust across the discount and the mortality associated with progressive disease after treatment. The scenario analysis generated comparable ICER per QALY associated with RD relative to VD ($120,974) and VCD ($117,191), therefore supports the robustness of base case analysis.

Summary/Conclusions: The local data-based health economic model estimates that RD could gain longer PFS and OS with acceptable cost-effectiveness, when compared to VD and VCD in Chinese mMM patients.

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. Aim: To describe 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 secondary immunodeficiency. 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 ovarium that was frozen by vitrification method. Reimplantation was successfully collected and reimplanted in 6 patients studied 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

E1476 DEVELOPMENT OF A NEW HAEMATOLOGICAL MALIGNANT PATIENT-REPORTED OUTCOME MEASURE FOR USE IN CLINICAL PRACTICE: HM-PRO

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Aims: The aims of this study were to identify issues important to patients with HM and development of a new patient reported outcome measure for use in daily clinical practice.

Methods: A conceptual framework was developed using preliminary literature search and discussions with physicians and patients. Patients with HM were then invited to produce a comprehensive item pool refined by a panel of experts. The generated items were then discussed in the data definition panel meeting to be included in the prototype version of the HM-PRO. Subsequently, a panel of experts and a panel of patients were asked to rate the items of the prototype HM-PRO for its language clarity, completeness, relevance and scaling followed by cognitive interviews with the patients to pilot test the HM-PRO.

Results: The preliminary literature search revealed that there is no PRO specifically developed for patients with HM for use in daily clinical practice. The conceptual framework comprised of two main themes: Qol (impact); and symptoms. 129 patients (male=78; mean age=61.1 years; SD=15.3; median age=64.9 years; age range=18-88 years; diagnosis –AML, ALL, CML, MM, ANHL, NHL, HL, MPN, and MDS) with mean duration of the HM of 3.6 years (SD=4.3; and range= 19-days-23 years) from 5 haematology centres were interviewed to identify the issues important to HM patients. A prototype version of HM-PRO was developed after data definition panel meeting with 34 items in impact category (Part A) and 23 items representing disease symptoms (Part B). Nine panel members of experts and 7 members of patients, rated the items and discussed them for their language clarity, completeness, relevance and scaling to reach consensus. 60 patients (male=36; mean age=63.8 years; SD=16.61; median age=69.2 years; and age range=18-91 years) with mean duration of the HM of 4.9 years (SD=6.4; and range= 14-days-26 years) were recruited for the pilot testing where 34 of which were involved in cognitive interviews. 92% of the patients reported that the statements were easy to understand and all issues important to them were covered; 95% stated that they were able to respond spontaneously and expressed their willingness to complete the instrument during their visit to the clinic; 97% reported that the statements were easy to read; 98% did not wish to delete any item; and 88% did not think they needed to add any new items.

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.
follow-up was 9 months (0-14) and all patients were alive. Hormonal results were evaluable for 3 patients: 2 patients were in premature ovarian insufficiency. Prescription queries are resolved during this meeting. Deferred oral chemotherapy can be returned to pharmacy stock, minimising waste. Intravenous chemotherapy is pre-planned with authorisation on the day of treatment if the patient is fit to proceed.

Methods: In line to the care pathway, we focussed 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 93% of patients noted a reduction in wait times & 88% felt they spent more time in consultations as a result. All patients noted they spent more time with the nurse specialist & benefited from not attending pharmacy. All patients rated the service 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 the way patients are seen & assessed and minimised drug wastage, an issue that are re-seeding malignant cells is a problem still to be conquered. 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 93% of patients noted a reduction in wait times & 88% felt they spent more time in consultations as a result. All patients noted they spent more time with the nurse specialist & benefited from not attending pharmacy. All patients rated the service more efficient.

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Sickle cell disease

DISEASE SEVERITY AND SLOWER PSYCHOMOTOR SPEED IN ADULTS WITH SICKLE CELL DISEASE

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Background: Psychomotor slowing is common in children with sickle cell disease (SCD), but little is known about its severity in adult patients. While the primary risk factor for psychomotor slowing is stroke, there has been mounting evidence that cognitive impairment also occurs in patients without a history of overt or silent stroke. Risk factors for cognitive impairment in patients with SCD without stroke are, however, not completely known, particularly in relationship to the SCD genotype.

Aims: We conducted a cross-sectional study to quantify psychomotor slowing, measured with the Digit Symbol Substitution Test (DSST), a pencil and paper test of executive function, in relationship with disease severity in adult patients with SCD attending an outpatient clinic. We also examined whether demographic, behavioral, physiologic, and pathologic factors that are known to be related to SCD severity and cognitive function in other settings are also related to psychomotor speed in these patients.

Methods: Genotype was used to group patients with SCD (n=88, age: 36.3 years, 33 males) in “severe” (homozygous for the mutated sickle hemoglobin HbS [HbS/HbS]) or “moderate” groups (compound heterozygous for HbS with either HbC [HbS/C], or βthalassemia [HbS/β+]). Standardized DSST scores based on published norms were used to define mild cognitive impairment, defined as ≤1.5 standard deviations (SD) below the DSST T-score (t-scores had a mean of 50 and SD of 10). Data on demographics, hematological parameters, hydroxyurea and opiate intake, stroke (including silent cerebral infarcts (SCI)) and transfusion history were collected concurrently with DSST. Analyses were repeated after exclusion of patients with a history of stroke (n=12). Age-adjusted p-value was calculated with logistic regression for all variables except age (unadjusted) and DSST T-score (already adjusted for age, sex and education in calculation of T-score).

Results: Among our patients, 56 (63%) had a “severe” genotype and 32 (27%) had a “moderate” genotype. Mild cognitive impairment was detectable in both the “severe” and the “moderate” group (30% and 9%, respectively, age-adjusted p=0.15). Compared to the “moderate” group, those in the “severe” group had significantly lower DSST scores (age, sex and education adjusted p values=0.006). Independent of adjustment for factors that differed between groups: hemoglobin, ferritin, hydroxyurea use, blood pressure parameters and stroke history. Results were similar after excluding patients with stroke.

Table 1. Predictor variables of interest

<table>
<thead>
<tr>
<th>Predictor variable of interest</th>
<th>“Severe”</th>
<th>“Moderate”</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>
<td>1.00</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.1 (1.8)</td>
<td>13.2 (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>47.6 (14.5)</td>
<td>51.0 (13.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>O2 Saturation (%)</td>
<td>97.5 (1.8)</td>
<td>98.1 (1.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>WBC count (X 10⁹/L)</td>
<td>9.7 (3.8)</td>
<td>9.2 (3.7)</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 10⁹/L)</td>
<td>344.1 (179.8)</td>
<td>263.3 (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 (IU/L)</td>
<td>32.2 (14.2)</td>
<td>269.2 (149.1)</td>
<td>0.19</td>
</tr>
<tr>
<td>Ferritin (mg/dL)</td>
<td>114.5 (1864.4)</td>
<td>404.3 (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>
</tr>
<tr>
<td>SBP (mm/Hg)</td>
<td>111.3 (13.4)</td>
<td>118.3 (13.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>68.8 (7.7)</td>
<td>73.5 (18.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP (mm/Hg)</td>
<td>83.1 (8.4)</td>
<td>88.6 (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. P1 includes SBI

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.

E1482 MONITORING OF CHRONIC HEPATIC DAMAGE IN SICKLE CELL DISEASE: LONGITUDINAL OBSERVATION OF A COHORT OF ADULT PATIENTS

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Background: Acute vaso-occlusive events (VOCs) in 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 for their entire lifetime. Therefore both SCD itself and related therapies may lead liver 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, cholesterol, liver synthetetic capacity, iron overload, viral hepatitis and hemolytic index), TE and liver imaging (ultrasound, MRI-R2*).

Results: 37 adult patients were evaluated: 32% HbSS, 68% HbSBβ+; mean 39.5yrs, 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 (R=0.43, p=0.008), ALT (R=0.42, p=0.001), AST (R=0.49, p=0.0022), conjugated bilirubin (R=0.59, p<0.001), ALP (R=0.51, p=0.002); a positive strong correlation was observed between TE and GGT (R=0.79, p<0.001), negative moderate correlation with the albumin (R=0.47, p=0.0048). We found that the group of patients on eritroexchange programmes had a value of stiffness lower than the group transfused (p=0.007). No significant difference was found between stiffness and LiC (R=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 (R=0.43, p=0.037), AST (R=0.54, p=0.006), conjugated bilirubin (R=0.52 values 0.009) and positive- strong correlation with GGT (R=0.68, p=0.001); these parameters except of ferritin (Rp=0.3, p=0.15) and AST (Rp=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 Rp=0.64, p=0.004; ALP Rp=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, HbSSβ+; 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.
Summary/Conclusions: Early identification of chronic hepatic disease sometimes pauci-symptomatic in terms of VOCs but able to lead to advanced stage and progressive fibrosis is crucial for suitable clinical management to avoid cirrhosis in SCD patients. The combination of TE with specific serum markers (GGT, ALP, albumin) is a valid tool to early detection of sickle hepatopathy.

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 (p=0.0187) and the HbF (p=0.0775). More specifically, the odds of retinal thinning as (p-value<0.10). However, the most predictive variables for retinal thinning as compared with normal controls (p<0.0001). SCD eyes with patchy retinal thinning have patchy areas of macular thinning on SD-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 retinal thickening in the central, inner nasal and outer temporal 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). Multivariate analysis showed that most predictive variables for retinal thinning as assessed after multivariate regression analysis were the need of chelation (p=0.0187) and the HbF (p=0.0775). More specifically, the odds of retinal thinning is 94.2% lower when chelation is present, and the odds of retinal thinning decreases by 12.9% when HbF increases by 1 unit.
Summary/Conclusions: In this study SCD eyes of all patients showed both inner and outer retinal thinning in the central and temporal macula. Ischemia caused by chronic occlusion of the deep and superficial capillary plexus could explain the different retinal layers’ damage and the pattern of thinning. No major statistics differences were found between the three sickle genotypes because of different value of retinal thinning area and age. SCD retinal complications are common and cause visual loss, but no major complications were revealed in our study. This may be due to the young age of our patients.

E1484
NON ABLATIVE TRANSPLANT CONDITIONING WITH TRESOLSULFAN IS CURATIVE IN A MURINE MODEL OF SICKLE CELL DISEASE
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Aims: Optimize non-myeloablative conditioning in a murine model of SCD that allows for sufficient donor RBC chimerism. Methods: Control (AA) and SCD (SS) animals were treated with varying conditioning regimens (+/- rescue with AA or SS marrow), including Tresolusufan (2-5g/kg), ACK2 (100-500ug/kg), anti-CD40L, and low-dose radiation, alone or in combination. Short and long-term toxicities, including survival, were monitored over a 12 month period. Hematologic effects were determined by assaying CBCs, reticulocytes, bone marrow (BM) cellularity and RBC chimerism (iso-electric focusing). Myeloid/lymphoid chimerism was monitored by FACS combined with droplet-digital PCR. Renal tubular function was assessed by measuring urine osmolality, and morbid animals underwent necropsy to assess organ damage.
Results: Erythroid hyperplasia was noted in the BM of SS, relative to AA mice. Tresolusufan, in a dose-dependent manner, decreased BM cellularity and induced cytopenia in AA and SS mice. AA mice were able to tolerate Tresolusufan at non-myeloablative doses of 6g/kg. In contrast, SS mice were unable to tolerate doses of 3g/kg unless RBC transfused by +3. At 3g/kg dose, erythroblast engraftment was transient in SS transplanted mice and most often absent by 2 months post-transplant, with only 25% of animals having sustained RBC chimerism at one year. In SS mice achieving 50% AA in peripheral blood, fertility was preserved, and repeated transplantation with partially matched controls, ACK2, anti-CD40L, or low-dose radiation, in combination with Tresolusufan (3g/kg), failed to improve engraftment. In contrast, increasing Tresolusufan to 3.6g/kg resulted in donor-erythroid chimerism at 3 months post-transplant in all mice, with improvement in hematologic parameters and normalization of hypertension. These results are currently being observed for fertility, organ toxicity and survival.
Summary/Conclusions: SCD mice closely mimic human disease in phenotype and ablative conditioning intolerance. Tresolusufan, at sub-myeloablative dosing, sustained erythroid chimerism and reversed the SCD phenotype. Our data suggest that pre-transplant conditioning with Tresolusufan alone may be permissive for engraftment, in an allogeneic and gene-corrected autologous clinical transplant setting.

E1485
SILENT CEREBRAL ISCHEMIA AND THROMBOEMBOLIC EVENTS IN SICKLE CELL DISEASE: ANALYSIS OF COAGULATION PARAMETERS AND MICROSTRUCTURAL ANALYSIS USING OPTICAL COHERENCE TOMOGRAPHY
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Background: The complications of Sickle Cell Disease (SCD) include stroke and silent cerebral infarcts (SCI). The increased incidence of thromboembolic events in SCD has only recently been recognized. Apart from red cell sickling other pathogenetic mechanisms have been proposed but they have not been clarified completely. Coagulation factors have been analysed in several studies in SCD but very limited data exist about global coagulation assays such as thromboelastography, which evaluates the contribution of platelets, coagulation factors and cellular elements in clot formation.
Aims: The aim of the present study was to assess the incidence of cerebral ischemia and TEEs in SCD patients and to investigate their pathophysiology with analysis of coagulation parameters, including thromboelastography.
Methods: 61 adult SCD patients were included in the study and underwent brain MRI. Measurements of fibrinogen, D-Dimers, antithrombin III, proteins S and C were performed (SIEMENS BCS) and thromboelastography ROME was performed in order to analyse NATEM CT (Closure Time), MCF (Maximum Clot Firmness), EXTEM CT, MCF and FIBTEM CT, MCF. Brain imaging as well as all clotting assays were performed in steady state and not during the course of an acute thrombotic or ischemic event.
Results: The median age of the patients was 51 yrs (range 27-70), 40 of them were female and 21 male. Abnormal findings were revealed in the brain MRI of 39/61 patients (64%), 13 of them had silent stroke, 7 patients had visible stroke and 4 patients had post-stroke sequelae. Only 5/55 patients had a previous history of overt stroke, 1.5 TIA. In the remaining 30/55 patients ischemic lesions were considered SCLs, in the absence of neurologic manifestations. In 3/5 patients with a previous stroke the size of the infarcts in brain MRI was larger (with maximum diameter up to 4.5 cm). 14/61 patients had a previous history of venous TEE (23%), in 7/14 the event was pulmonary embolism and 2/14 had recurrent TEE. 14/81 (23%) of patients had a previous history of acute chest syndrome (ACS). In total 48/61 patients (78.7%) were already on treatment with hydroxyurea when they underwent the analysis. Elevated platelets were found in 22/61 patients (36%), elevated fibrinogen in haematologica | 2017; 102(s2) | 805
Madrid, Spain, June 22 – 25, 2017
4/51 (1.6%), positive D-Dimers in 57/59 (96.6%), decreased protein S in 10/61 (16.3%) and decreased protein C or 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, and 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, and p=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 potential 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 INFLUENZA TYPE B VACCINATION
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1Royal Free Hospital, London, United Kingdom, 2Medical Research Council Unit Gambia, Fajara, Gambia, 3Imperial College London, London, United Kingdom

Background: Infection causes significant morbidity and mortality in patients with sickle cell anemia, especially in populations without reliable access to antimicrobial prophylaxis and treatment. The long-standing 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 incidence 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 2012 and April 2013). Patients were divided into two groups; Group 1: Patients who had a painful crisis during the study (41 patients, mean age: 11.5 years) and Group 2: Patients who were in steady state during the study (30 patients, mean age: 11 years). Blood samples were taken from the patients for complete blood count, serum levels of C-reactive protein (C-RP), interleukin-1 β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IGF-1, IGFBP-3 and IGFBP-3 gene expression.

Results: When the patients in both groups were compared in terms of serum IGF-1 level; serum IGF-1 levels were normal in all patients (100%) in group 2 and 33 patients (80.5%) in group 1, and the difference was considered to be statistically significant (p <0.001). Also, when the patients were examined for IGFBP-3 level; serum IGFBP-3 level in Group 2 was found to be significantly lower in Group 1 (p <0.001). Also, when the patients were examined for IGF-1 and IGFBP-3 gene expression, no significant difference was found between the groups (Table 1). A negative correlation was found between leucocyte level and IGF-1 in group 1, and IGF-1 gene expression and CRP in group 2. Serum IGFBP-3 and IL-6 levels were found to be significantly lower in patients without any painful crisis than those with painful crisis in the last year (p <0.05).

Table 1.

E1488
THE ASSOCIATION OF IGF-1 AND IGFBP-3 SERUM LEVELS AND GENE EXPRESSION WITH THE PATHOGENESIS OF INFLAMMATION IN SICKLE CELL DISEASE
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Background: Sickle cell disease (SCD) is one of the chronic inflammatory diseases; Serums markers of inflammation have provided evidence for a state of chronic inflammation in sickle cell disease (SCD). Inflammation promotes endothelial adherence to sickle erythrocytes.

Aims: We aimed to investigate the serum insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3) levels and gene expression in the pathogenesis of inflammation in sickle cell disease and to determine its role in painful crises.

Methods: A total of 71 patients aged 2 to 18 years, who were followed with the diagnosis of SCD in our department, were included in the study between April 2012 and April 2013. Patients were divided into two groups; Group 1: Patients who had a painful crisis during the study (41 patients, mean age: 11.5 years) and Group 2: Patients who were in steady state during the study (30 patients, mean age: 11 years). Blood samples were taken from the patients for complete blood count, serum levels of C-reactive protein (C-RP), interleukin-1 β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IGF-1, IGFBP-3 and IGFBP-3 gene expression.

Results: When the patients in both groups were compared in terms of serum IGF-1 level; serum IGF-1 levels were normal in all patients (100%) in group 2 and 33 patients (80.5%) in group 1, and the difference was considered to be statistically significant (p <0.001). Also, when the patients were examined for IGFBP-3 level; serum IGFBP-3 level in Group 2 was found to be significantly lower in Group 1 (p <0.001). Also, when the patients were examined for IGF-1 and IGFBP-3 gene expression, no significant difference was found between the groups (Table 1). A negative correlation was found between leucocyte level and IGF-1 in group 1, and IGF-1 gene expression and CRP in group 2. Serum IGFBP-3 and IL-6 levels were found to be significantly lower in patients without any painful crisis than those with painful crisis in the last year (p <0.05).
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 effects of inflammation, 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
The plasmatic hemoglobin (cell-free hemoglobin - Hb) was measured by using (Bantu / Bantu + HC) and 54 without (Bantu / Bantu - HC), respectively.

Methods:

Aims:

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 a 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 national newborn 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; Rola 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 Oncohematology Pediatric Association (AIEOP). The panel has rigorously revised the literature (from 1990 to 2016), the existing recommendations/guidelines of other countries where newborn screening already exists for SCD, and the SCDADE system (SCD Assessment, 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 newborns 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 new born 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 principals focus of current research. The SCA, a homozygous condition for Hb S, is a hereditary haemolytic anemia 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 progress 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 (G) of the NPRL3 gene plays a protective role at hemolysis in individuals with SCA, suggesting this variant as a genetic marker of hemolysis.

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 according to their hemoglobin values (Hb): (HC) 22% of the total value 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 covariables. Statistical software was used and assumed p <0.05 as significant

Results: Evaluating the recessive model (GG / GT versus TT), we found a significant difference between the different genotypic patterns (p=0.026), and not significant for the dominant model. Therefore we performed an analysis for the normalization of SNP in the variation of cell-free Hb levels and hemolysis markers commonly used as hemolysis parameters (relative reticuloocytes, the enzymes lactate dehydrogenase and aspartate aminotransferase and unconjugated billirubin), and we found that the individuals genotypic profile was responsible for 50.7% of the Hb variation (Wilk’s λ: 0.497, F: 4.08, p-value <0.001), suggesting that the SNP may play a role in characterizing the hemolytic profile of our patients with SCA.

Summary/Conclusions: The SNP here studied is located in the intronic region of the NPRL3 gene, where the main regulatory elements of the alpha globin gene cluster (HS-48, HS-30 and HS-33) are also found. Studies have already suggested that the protective effect of the G allele of the SNP on the hemolytic score levels is probably related to the role of this genetic variant in the expression of the alpha globin genes. Its promising that aditional analyzes in other ethnic groups and models of hemolytic anemias, such as those of an acquired character are realized. This is one of our next step in the attempt to suggest this variant as a genetic marker capable of assisting in the characterization of the hemolytic and prognostic profile of people with SCA.
**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.

**E1494**

**REDUCED SERUM HAE莫PEXIN LEVELS IN HAE莫GLOBIN SC DISEASE OCCUR INDEPENDENTLY FROM THE DEGREE OF HAEMOLYSIS**

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**Background:** 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 haemopexin 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 (Vinchi 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.

**Aims:** 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).

**Methods:** 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), and haemopexin (Abcam) levels. Statistical analysis was performed with GraphPad Prism v5.0 and data are expressed as mean±standard deviation.

**Results:** As expected, serum LDH, total and indirect bilirubin, and reticulocyte counts were increased in HbSC patients (P<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=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), and considering World Health Organization definitions of anaemia for men (Hb below 13 g/dL) and women (Hb below 12 g/dL), 20 (50%) patients in our HbSC cohort were anaemic, thus fulfilling criteria for compensated haemolysis. HbSC patients with compensated haemolysis were not significantly different from their anaemic counterparts, with similar reticulocyte counts, LDH, bilirubin, haemoglobin (9.83±6.98 vs 7.73±3.813 ng/dL, P= 0.10), and total haem levels (33.92±2.4 vs 37.55±2.9 μM, P=0.30). We also found an unexpected negative correlation between haemoglobin and haemopexin, r=-0.42 (Pearson), P=0.007.

**Summary/Conclusions:** Despite the putative importance of reduced haemopexin in the pathophysiology of sickle cell disease, HbSC patients do not always present with haemopexin deficiency, regardless of the intensity of the haemolytic state, and possibly to due to a lesser importance of intravascular haemolysis. Our data support that non-anaemic HbSC patients may be equally affected by haemolysis, but intravascular haemolysis does not predominantly regulate haemopexin production. We suggest that excessive free haem and low haemopexin probably represent a lesser contribution to the pathophysiology of complications found in this subgroup of sickling disorders.
Stem cell transplantation - Clinical

E1496
HIGH PROGNOSTIC VALUE OF PRE-SCT MOLECULAR MINIMAL RESIDUAL DISEASE ASSESSMENT BY WT1 GENE EXPRESSION IN AML TRANSPLANTED IN CYTLOGIC COMPLETE REMISSION
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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 Abelson-positive metaphases) and before allo-SCT (81/122=66% MRD-WT1-negative and 41/122=44% 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 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 = 19.9, 95% confidence interval [95% CI] 2.9-120.7; 8.3 DFS log-rank p<0.0001; HR=7.3, 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 the use of more aggressive immunosuppression regimens.

E1497
GOOD IMMUNOLOGICAL RECONSTITUTION IN ADULTS WITH ACUTE LEUKAEMIA AFTER ALFA-BETA TCR/CD19+ DEPLETED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)
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Background: Haploidentical transplantation 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 immune reconstitution to improve clinical outcomes separating graft rejection from GVHD. Favourable donor immune responses. Selective elimination of αβ+ T cells retains in the graft NK, dendritic cells, monocytes and γδT lymphocytes. Graft versus host disease (GVHD) is a complication of allo-SCT occurring when donor T lymphocytes recognize host tissue antigens as foreign. Two major types of GVHD are acute GVHD (aGVHD) and chronic GVHD (cGVHD). GVHD is caused by the immune reaction of donor T lymphocytes against host tissues. The clinical manifestations of acute GVHD include skin, gastrointestinal tract, liver, respiratory, and other organ involvement. Chronic GVHD is a clinically and histopathologically distinct complication that may develop in patients who have received allo-SCT.

Methods: Aims: With the aims of confirming these results in adults, we tested this approach in adults 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 immune reconstitution to improve clinical outcomes separating graft rejection from GVHD. Favourable donor immune responses. Selective elimination of αβ+ T cells retains in the graft NK, dendritic cells, monocytes and γδT lymphocytes. Graft versus host disease (GVHD) is a complication of allo-SCT occurring when donor T lymphocytes recognize host tissue antigens as foreign. Two major types of GVHD are acute GVHD (aGVHD) and chronic GVHD (cGVHD). GVHD is caused by the immune reaction of donor T lymphocytes against host tissues. The clinical manifestations of acute GVHD include skin, gastrointestinal tract, liver, respiratory, and other organ involvement. Chronic GVHD is a clinically and histopathologically distinct complication that may develop in patients who have received allo-SCT.

Results: Grafts contained a median of 11x106/kg (range 5-19) CD34+ cells, 4.3x106 CD3+ T cells/kg (range 1-36), 4.9x105/kg (range 0.4-62) αβ+ T cells, 4.1x106 CD56+NK cells/kg (range 1-34), 5x104 B cells/kg (range 1.5-32) and 22x106 CD56+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 (3.7x104/kg) and the second one affected by 6GPDH deficiency experienced a late onset hepatic GVHD. Eight patients had skin limited grade II/IV GVHD that required short course steroids. Only two patients have so far developed mild GVHD 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 was confirmed in all patients. CMV viremia was controlled with ganciclovir in the majority of the patients. CMV reactivation occurred in 15% (4/12 in relapse, 4/20 in CR), 13 patients survive at a median follow-up of 29 months (range 5-55).

Summary/Conclusions: The infusion of αβ/CD19-depleted grafts confirmed a fast immunological reconstitution also in adults. Relapse is still a major concern in patients already in relapse at transplantation.

E1498
UNMANIPULATED HAPLOIDENTICAL TRANSPLANTATION CONDITIONING WITH BUSULFAN, CYCLOPHOSPHAMIDE AND ANTI-THYMOCYTOGLOBULIN FOR ADULT SEVERE APLASTIC ANEMIA: GOOD OUTCOME AND PROGNOSIS ANALYSIS
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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. Under this approach, a rapid immunological reconstitution and very promising outcome have been reported in pediatric patients. The patients’ median age was 25 years (ranging from 18 to 45). All of 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 a feasibility study of the full outpatient conduct of E1500 al blood stem cells, ROSIS employing autologous non-cryopreserved peripheral blood stem cells were higher after G-CSF mobilization, the subset of phenotypic stem cells was 31% CD34+ cells were collected. PFX was administered twice in 10 donors. Side effects CTC grade 2 occurred in 39% of donors and included gastrointestinal reactions. Median cell numbers assessed in 10 G-CSF-mobilized recipients were used as controls.

Results: Criteria for feasibility were met as in 22 out of 23 donors ≥2x 10^6/kg CD34+ cells were collected. PFX was administered twice in 10 donors. Side effects CTC grade 2 occurred in 39% of donors and included gastrointestinal responses. The unmanipulated grafts were infused according to local protocol and subsequently rituximab (100mg/kg every 2 months over a 12-month period). The cumulative dose of Cy is 200mg/kg.

Results: 80 females and 51 males were included; median age was 47 years. 26 have PMMS, 42 RRMS, and 61 SPMS. 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 1x106/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 at 1.4% and 109L absolute granulocytes 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 conduct. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

E1501

VEDOLIZUMAB IN STEROID REFRACTORY INTESTINAL GASTRO-VESUS-HOST DISEASE

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Background: Steroid refractory intestinal graft-versus-host disease (GVHD) is a common 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 adhesion 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.

**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 allogeneic 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 allogeneic SCT.

**Aims:** In this analysis, we aimed to elucidate the factors that affect the outcomes of patients with autologous GVHD.

**Methods:** This was 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 histo-pathological 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 myeloma (14 patients, 73.7%) and the most common conditioning regimen used was melphalan (16 patients, 84.2%). The median age at ASCT was 61.9 (range 49-72.6) years and the median time from disease diagnosis to ASCT was 3.1 (0.3-9.6) years. The median number of prior therapeutic regimens was 2 (range 1-7). GVHD manifested with gut involvement in all 19 patients, skin involvement in 8 patients (42.1%) and liver involvement in 2 patients (10.5%). The median time to symptom onset was 11 (range 3-80) days and the median time to GVHD diagnosis was 12 (range 2-162) days. Most patients (14, 73.7%) had grade 3 or 4 GVHD and the clinical grading correlated with the histopathologic grading in all patients. 8 patients received steroids with an average dose of 0.6-2.2mg/kg prednisone equivalents. The median time to symptom resolution was 15 (range 3-162) days and 14 patients (73.7%) achieved a complete resolution of symptoms. The median overall survival (OS) from the time of ASCT was not reached and 53% of patients were alive 3 years after ASCT. Of the 19 patients diagnosed with autologous GVHD, 5 (26.3%) died due to complications of GVHD or its treatment. Delay in initiation of steroids beyond 1 week was associated with lower response rates to treatment (30.8% vs 69.2%, p=0.03), longer duration of symptoms of GVHD (median 28 vs 4 days, p=0.02), and a trend towards worse 1-year OS (64.5% vs 83.3%, p=0.1). Higher steroid doses were associated with a trend towards better complete response rates (76.9% vs 23.1%, p=0.5), although this difference did not reach statistical significance.

**E1503**

**CNS DEMYELINATION AFTER HAPLO-HSCT AND ITS ASSOCIATION WITH THE IGG INTRATEHEL SYNTHESIS INDEX AND ANTI-MYELIN OLIGODENDROCYTE GLYCOPROTEIN ANTIBODY IN CEREBROSPINAL FLUID**

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**Background:** Haploidentical haemopoietic stem cell transplant (haplo-HSCT) is an upfront and effective therapy for haematological patients, but it usually has many complications such as neurological complications. As one of the neurological complications following haplo-HSCT, immune-mediated demyelinating diseases of the central nervous system (CNS) seriously affect the patient quality of life. However, the incidence, risk factors and pathogenesis of CNS demyelination are not very well understood.

**Aims:** To analyse the incidence, risk factors, and prognosis of CNS demyelination after haplo-HSCT.

**Methods:** A study was conducted in 1,526 patients who underwent haplo-HSCT between January 2013 and June 2016. The definition of CNS demyelination during haplo-HSCT was confirmed by neurologic signs, MRI abnormality corresponding to the neurologic signs, abnormal CSF studies and the presence of systemic GVHD or the response to immunosuppressive therapy (Grauer O et al. Brain. 2010; 133(10): 2852-2865, Chronic graft versus host disease.
Page 243-51, 2009, Thomas’ Hematopoietic Cell Transplantation, Page 766-75, Fifth Edition, 2016, Polman C H et al. Ann Neurol. 2011; 69(2): 292-302. Patients who did not meet these criteria and were determined to have CNS infection (bacterial, fungus, and viruses), neurotoxicity or malignancy relapse, based on clinical and laboratory findings, were excluded. The CSF immunoglobulin index includes BBB permeability, the IgG index, the CSF IgG intrathecal synthesis index, the CSF myelin basic protein index, CSF and blood anti-myelin basic protein antibody, CSF and blood anti-myelin oligodendrocyte glycoprotein antibody.

Results: Thirty patients developed CNS demyelination after haplo-HSCT. The cumulative incidences of the diseases at 100 days, 1 year and 2 years post transplant were 0.6%, 1.6% and 2.3%, respectively. The mean age at the time of presentation was 26.5 years (range, 10-52 years), and the mean time from transplant to the onset of neurologic symptoms was 216 days (range, 17-844 days). Nineteen patients received a corticosteroid pulse, five patients received immunoglobulin, and six patients received supportive treatment and an immunosuppressive treatment in immunosuppressive symptoms improved in all patients. The mean duration from the time of improvement to deterioration was 5 days (±4). In univariate analysis, we found that BBB permeability and the CSF IgG intrathecal synthesis index were related to the occurrence of CNS demyelination (p<0.1). In multivariate analysis, the CSF IgG intrathecal synthesis index (OR=1.017, 95% CI: 1.003-1.031, p=0.019) and CSF myelin oligodendrocyte glycoprotein antibody (OR=12.059, 95% CI: 1.141-127.458, p=0.038) were independently associated with the onset of CNS demyelination. We also studied the possible pathogenesis of CNS demyelination. Immune reconstitution (the cell proportion of CD19+B cells, CD3+ T cells, CD4+ T cells), the count of leucocytes, lymphocytes, monocytes and platelets, as well as the level of immunoglobulins A, G, and M, were measured on days 30, 60 days, and 90 days after HSCT showed no significant differences between CNS demyelination and no demyelination (p>0.05). The probabilities of overall survival showed no significant differences between patients with and without demyelination.

Summary/Conclusions: The CSF IgG intrathecal synthesis index and CSF anti-myelin oligodendrocyte glycoprotein antibody are independently risk factors for the onset of CNS demyelination after haplo-HSCT and have no influence on long-term survival. Immune reconstitution may not be pathogenesis of CNS demyelination.

E1505
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.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rdCR (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 conditioned with reduced intensity regimen. Stem cell source was HLA-identical sibling in 16 (20%), haploidentical (haploID) in 24 (26%) and alternative donor in 50 (54%). Median follow-up was 64 months (95% CI 39.8-88.2 months). A positive MFC MRD was defined by the presence of at least 1x10^3 residual leukemic cells at four or eight (since 2011) color flow-cytometry. WT1 copy

Results: 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.

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, BMT procedure, donor chimerism MFC and relapse incidence (p<0.03 for MFC based MRD) and CR status at BMT (better for CR2, p<0.05). Specifically patients transplanted in a MRD negative status had comparable OS irrespectively of ELN at diagnosis (2-years OS of 62.2% and 52.7% among MFC MRD negative patient with ELN risk low or intermediate/high, respectively, Fig.1). The predictive value of MRD resulted independent from all other analyzed variables, although patients with positive MRD undergoing HAPLO BMT had a slightly better outcome. Multivariate OS analysis revealed that MRD status (evaluated by any method) was the only independent predictor of OS (p<0.05 for both). Pre BMT MRD was also a strong predictor of cumulative incidence (CI) of relapse in competitive risk analysis (p<0.01 and p<0.03, respectively, for WT1 and MFC MRD). Multivariate CI of relapse analysis showed that donor source and MRD significantly influenced relapse risk (p<0.05 and <0.01, respectively).

Aims: There are limited data concerning the impact of specific HLA mismatches on patient outcome and we therefore interrogated the EBMT database in order to characterize the impact of mismatch on transplant outcome.

Methods: 937 patients with AML in CR1 or CR2 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-DO and 74 mismatched at HLA-DPB1. 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 26.0% and relapse incidence 24%. The 2 year LFS was 47% and OS 40% for patients treated with HAPLO BMT. There were no significant differences between patients transplanted with HAPLO or HLA matched donors for any of the clinical and transplant related variables.

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-parametric detection to evaluate disease status. Patients were given IFN-α-2b 3 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 transfrom negative to positive, or dynamic increasing copy number of WT1 more than 200 copies/10^4 abl, or decreasing of donor chimerism(≤ 90%).

Results: In our study, 31 patients received IFN-α-2b pre-emptive therapy, and 67 patients received non-IFN-α-2b therapy as such as: withdrawal immunosuppressant, traditional DLI or DC-CIK immunotherapy. 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-αtreatment was 60 days (range: 5–720 days), Twenty five patients had responded to the treatment without progression 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 60 patients who received non-IFN-α-2b therapy the response rate 64.5% (33/51) patients were responders 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 groups (P=0.000). 31 patients of IFN group tolerate well and no patient terminated therapy due to side effects. Among them, 31 patients received IFN-α-2b pre-emptive therapy, and 67 patients received non-IFN-α-2b therapy as such as: withdrawal immunosuppressant, traditional DLI or DC-CIK immunotherapy. 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-specific survival rate (LSS) were 52.6% and 44.6% respectively.
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 for disease progression post allo-HSCT. Further large-scale investigation is warranted.

E1508
PREDICTING SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION. THE GATMO SCORE
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Background: Several attempts to predict mortality after autologous stem cell transplantation (ASCT) have been made, like Hematopoietic Stem Cell Transplant Comorbidity Index (HCT-CI) score, originally described by Sorror for allogeneic HSCT. There is no score applicable to the clinical practice that integrates comorbidities with other patient characteristics.

Aims: To describe a comprehensive score that combines comorbidities with other factors and analyse the impact of this score in OS and NRM after ASCT in a cohort of patients transplanted in Argentina.

Methods: We retrospectively reviewed a cohort of 1453 medical records of adult patients who received an ASCT in our centres between October 2002 and August 2016, for Multiple Myeloma or Lymphoma. We compared NRM and Relapse with CI, OS with KM and long term MVA with fine-Gray or Cox regression. We included in the score all the factors that remained significant after MVA for NRM, and assigned a score of 1 if the Hazard ratio (HR) was around 2 (1.5-2.5) and 2 if it was around 3 (2.6-3.5).

Results: Mean age was 50.7 years (range 15-74); 57% were male, 52% had Multiple Myeloma, 29% Non Hodgkin Lymphoma and 19% Hodgkin Lymphoma. 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), 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, 469 (32%) 1, 381 (26%) 2, 241 (17%) 3, 137 (9.5%) 4 and 47 (3.5%) ≥5. The hazard ratio for NRM increased proportionally with the score. This observation should be confirmed in larger series.

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.

E1509
A RETROSPECTIVE ANALYSIS OF PATIENT CHARACTERISTICS AND RISK FACTORS FOR ADMISSION TO THE INTENSIVE CARE UNIT (ICU) FOLLOWING HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (HDC-ASCT)
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Background: HDC-ASCT is a standard treatment modality for patients with myeloma and lymphoma. It carries a low, but significant risk of morbidity and mortality. Given that the upper age limit for patient selection continues to increase, it is important to have an objective way of assessing patient suitability for HDC-ASCT. Admission to the ICU is an ominous clinical event post HDC-ASCT 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.

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 GraphPad 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 neutropen count <1×10⁹/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; inotrope support, 2; haemofiltration; 1) and 2 required only management of...
fluid balance. Six patients required multi-organ support (non invasive ventilation/infusion, 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 accidents (1), and multifocal hepatic necrosis (1) versus host disease (2). 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 has an EF <50% and 2 died 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 was neutropenic fraction 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, Etoposide, 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).

**Results:** All patients had chemo-sensitive disease and before transplantation, 34 patients (83%) were in complete (CR) and 7 (17%) in partial remission (PR). A median number of 4,2*10^6 CD34+ cells/kg (range: 1.60-13.30) were infused. All patients showed engraftment with a median time to achieve an absolute neutrophil count >1*10^9/L of 10 days (range 8-13) and to platelets >20*10^9/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 were transfused. The median duration of hospitalization was 25 days. The most common grade 3 and 4 toxicities during the whole treatment period were 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%) (range 2-29 months). Until today nine patients received an additional allogeneic transplantation.

**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 COMITANT 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 endoscopy 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 endoscopy; 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-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% (P<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 (P=0.015) 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, there has been, 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...
CFBF-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)(p13.1q22). Disease status at HSCT was CR1 in 19 (31%) and CR2 in 42 (69%). The most frequent abnormality was trisomy 8 (n=11), followed by t(5;18), t(1;11), t(1;8), t(1;19), -Y, and internal control (ABL1) transcript levels were detected simultaneously and quantitative results were expressed as the percent ratio of fusion to ABL1 transcript levels (fusion/ABL1)×100.

Results: MRD by RT-PCR at HSCT was evaluable in 43 patients (70%) and 36 of 44 (84%) had evidence of MRD (MRDpos). RT-PCR was <0.1% in 22 patients, ≥0.1% and <1% in 7 and ≥1% in 8 patients. Overall survival (OS) and leukemia free survival (LFS) at 4-years was 100% and 85.7% in MRDneg and 65.4% and 61.6% in 37 MRDpos patients respectively (p=0.09 and p=0.3). The incidence of disease progression was comparable between MRDneg and MRDpos patients, 15% vs 16% at 4 years. There was no increase in the risk of progression with higher levels of MRD by RT-PCR (p=0.6). None of the other variables were prognostic for OS, LFS and disease progression. There was no transplant-related mortality observed in MRDneg group while the incidence was 22.6% at 2 years in MRDpos group. Summary/Conclusions: Durable complete remissions can be achieved in CBF AML patients with HSCT even if they are MRDpos at HSCT.

E1513
LONG-TERM OUTCOME OF ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT SEVERE APLASTIC ANEMIA WITH ABNORMAL CYTOGENETICS AT DIAGNOSIS

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Background: Cyto genetic 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. Clonal 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: The most frequent abnormality was trisomy 8 (n=11), followed by inversion 9 (n=2). Other CAs included t(1;3), t(5;18), t(1;11), t(1;8), 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 of non-relapse causes. In the first HSCT, the 5-year estimated OS rates were 94.7±5.1% in hypoplastic bone marrow with dysplastic cells was considered as hypoplastic 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.

E1514
PROGNOSTIC VALUE OF PET/CT PRIOR TO AUTOLOGOUS HCT IN RELAPSED / REFRACTORY LYMPHOMA

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Background: Positron Emission Tomography /Computed Tomography (PET/CT) is emerging as a powerful prognostic tool in the management of Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL). A number of retrospective single center cohorts have reported that a positive PET/CT prior to autologous Hematopoetic Stem Cell Transplantation (HSCT) is an adverse factor associated with higher relapse risk. However, important heterogeneity is noted in these studies due to differences in timing of PET/CT prior to HCT as well as different metabolic activity threshold (i.e. Deauville ≤2 vs ≤3). At our institution, we perform PET/CT within 4 weeks prior to HCT and after all intended salvage therapy is administered.

Aims: We sought to further investigate the prognostic value of PET/CT in relapsed / refractory lymphoma patients prior to HCT.

Methods: After due IRB approval, patients who received autologous HCT at our institution for relapsed / refractory lymphoma between 2010 - 2016 were identified. All variables were retrospectively extracted. PET/CT reports were reviewed and metabolic activity was assigned per Deauville criteria. Patients with primary CNS lymphoma were excluded. Refractory disease indicates disease progression prior to completing planned first line therapy. 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 rank tests. Competing events were computed using Grey’s method considering non relapse mortality as a competing event for relapse. Analysis was computed using JMP software, version 11.

Results: A total of 53 patients underwent HCT for relapsed / refractory lymphoma with 80% of the cohort having HL. Median follow up of the entire cohort 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...
was 109 days (55-395) vs 271 days (55-440) for PET positive vs PET negative patients, respectively. Mortality post relapse was very low with the remaining patients achieving long term disease control with immunotherapy alone (57%), allogeneic HCT (29%) and combination chemotherapy (14%). Median follow up of patients with long term disease control was 1093 days (177-1271). Causes of death post HCT relapse was progression of disease in all cases. Summary/Conclusions: Despite inherent limitations of this analysis, we present a number of important observations: 1. Deauville score ≤3 is an appropriate cutoff for metabolic activity pre-HCT and is associated with significantly decreased relapse and improved PFS. 2. PET positive status will better identify patients who may benefit from maintenance strategies post HCT. 3. Time to relapse for PET positive patients is significantly shorter highlighting the need for early initiation of pre-emptive maintenance therapy. 4. Long term disease control is possible in a high proportion of patients despite relapse post HCT. These important observations require further study.

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. However, the efficacy of allogeneic lymphoma-targeted immunotherapy (Chemo-DLI) remains controversial. Here we report the results of the first prospective study evaluating the safety and efficacy of 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 Chemo-DLI in patients who were MRD-positive after allo-HSCT.

Methods: We enrolled 115 consecutive patients who received either DLI (n=20) or Chemo-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 Chemo-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 (75%) and 47 (78.3%) patients in the DLI and Chemo-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 Chemo-DLI groups, respectively. The 2-year probabilities of disease-free, overall, and GVHD-free/relapse-free survival after preemptive interventions were 58.9% versus 54.3% (P=0.682), 69.3% versus 78.1% (P=0.361), and 44.4% versus 35.1% (P=0.489) in the DLI and Chemo-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 Chemo-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 1-year relapse rate in allo-HSCT. 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 78% (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 2.9) 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 3 to 4 acute 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).

Figure 1.

Summary/Conclusions: UCBT showed good OS with the low incidence of NRM even in patients with high HCT-CI scores. These results indicate that a single unit umbilical cord blood might be a promising stem cell source for patients with multiple comorbidities. Further studies are needed in order to validate these results.

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 collateral 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 with maternal or collateral (homozygous) disease (33%, P=0.51). Similarly, there was no significant difference in the cumulative incidences of grade 3 to 4 acute 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).

Figure 1.

Summary/Conclusions: UCBT showed good OS with the low incidence of NRM even in patients with high HCT-CI scores. These results indicate that a single unit umbilical cord blood might be a promising stem cell source for patients with multiple comorbidities. Further studies are needed in order to validate these results.
the new strategy. Trial registration: The study is registered at www.clinicaltrial.gov as NCT02412423.

Results: We found that low dose PT/Cy combined with ATG could alleviate GVHD in mice and could increase the number of Treg cells while have no effects on CD4+ or CD8+ T cells. A total of 40 patients with myelodysplastic syndrome (MDS) and leukemia undergoing haploidentical HCT from maternal or collateral donors were enrolled in the study. The cumulative, 100-day incidence of acute GVHD, grades II-IV, in Group A (17%; 95% CI, 5%–29%) was significantly lower than both that in Group B (33%; CI, 25%–41%; P=0.04) and that in Group C (56%; CI, 42%–70%; P<0.001). The 1-year probabilities of NRM (5%; CI, 0%–12%), OS (84%; CI, 69%–100%), and LFS (83%; CI, 70%–96%) in Group A were similar to that in Group B, but was significantly lower than that of Group C (28%; CI, 15%–41%; P=0.006; 65%; CI, 51%–79%; P=0.02; and 65%; CI, 51%–79%; P=0.04; respectively).

Summary/Conclusions: Low dose PT/Cy can enhance the protective effect of ATG/G-CSF on GVHD. Conditioning with ATG/G-CSF and low-dose PT/Cy might be a feasible option for patients undergoing HLA haploidentical, T-cell replete HCT, in particular for those with high GVHD risk.

E1518
HEPATITIS B REACTIVATION IN HEMATOPOIETIC STEM CELL TRANSPLANTED PATIENTS: 22 YEARS EXPERIENCE OF A SINGLE CENTRE

Background: Reactivation of inactive viruses is an important complication of haematopoietic stem cell transplantation (HSCT). Suggestion of strategies to combat this problem will probably decrease transplant related mortality and morbidity.

Aims: Aim of this study is to evaluate the clinical progress and risk factors for reactivation in HSCT patients who were infected with hepatitis B virus (HBV) with the prospect of developing recommendations for a better clinical care.

Methods: Patient files and electronic records of 561 patients who received HSCT between 1994 and 2015 at the Bone Marrow Transplantation Center of Cerrahpaşa Medical Faculty were retrospectively evaluated. A total of 66 patients with HBsAg (n=15; 12 autologous, 3 allogeneic) and anti-HBC IgG positivity (n=51; 29 autologous, 22 allogeneic) were included in the study. Cases were grouped according to transplant types (allogeneic or autologous) and anti-HBc positivity (isolated anti HBc IgG positivity) 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 HSCT. While cumulative incidence of reactivation was 7% at day 60, it went up to 16% and 44% at days 270 and 730 following HSCT, respectively. In Anti Hbc IgG positive group, allogeneic HSCT (n=22) was a higher risk factor for reactivation (31.8%) than autologous HSCT (n=29, 6.8%). Relative risk of reactivation in the allo-transplanted patients who were anti-HBC IgG positive and anti-HBs negative was 6.8 when compared to anti-HBC and anti-HBs positive patients (n=95, 55% vs n=13, 10%) (95% CI: 1.3–46.5). Cumulative incidence of reactivation in anti-HBc IgG positive anti-HBs negative patients (isolated anti HBC IgG positivity) was 11% at day 10 day, 33% at day 133, 50% at day 400 and going up as high as 75% at day 940.

Summary/Conclusions: The results of our study indicate that HBsAg positive patients undergoing autologous or allogeneic HSCT should receive prophylaxis at least one year posttransplant. Anti-HBc IgG positive patients carry the risk of reverse seroconversion, with receivers of allogeneic HSCT having higher risk than those of autologous HSCT. Patients who are anti-HBC IgG positive and anti-HBs negative should receive prophylaxis for HBV if allogeneic HSCT is to be performed. However, close follow-up seems to be acceptable rather than a prophylactic treatment for anti-HBc IgG positive patients undergoing autologous HSCT.

E1519
ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION FROM HAPLOIDENTICAL DONOR WITH POST-TRANSPLANT CYCLOPHOSHAMIDE WAS RELATED TO LESS INPATIENT COST COMPARED TO CORD BLOOD TRANPLANTATION

Background: The number of allogeneic HSCT from alternative donors such as cord blood (CB) and haploidentical 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 was 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 with PT/CY, and 48 with single-unit CB. The median initial inpatient cost was €49179 (IQR, 37010–68693), 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, €68852, P=0.008 vs CB), MUD (median, €39978, P=0.01 vs CB), and haplo (median, €39762, P=0.01 vs CB) (Figure). Also, the transfusion cost was highest in CB (median, €22750) compared with haplo (median, €12866, P=0.001 vs CB), MUD (median, €12699, P=0.001 vs CB), and MUD (median, €13118, P=0.001 vs CB). The median hospitalization days were 67 in CB, 61 in haplo (P=1.0 vs CB), 46 in MRD (P=0.001 vs CB), and 49 in MUD (P=0.01 vs CB). Among the clinical variables such as diagnoses (acute leukemia or others), refined disease-risk index (low/intermediate/high), donor source (MUD, MUD, haplo, or CB), age, first or second HSCT, intensity of conditioning (RIC or MAC), and both or without comorbidity, graft failure, GVHD III-IV, and admission to the intensive care unit (ICU), multiple regression models revealed CB (P=0.001), haplo (P=0.003), graft failure (P=0.001), admission to ICU (P=0.001), and MAC (P=0.05) were the factors that increased the initial inpatient cost. The transfusion cost was increased by CB (P=0.001), graft failure (P=0.001), admission to ICU (P=0.001), and MAC (P=0.001). CB (P=0.001), haplo (P=0.003), and GVHD III-IV (P=0.01) were selected as factors associated with longer hospitalization period.

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 partly attributed 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.

E1520
THE ROLE OF PPARβ EXPRESSION IN PATIENTS WITH GVHD FOLLOWING ALLOGENEIC HSCT

Background: The acute graft versus host disease (aGVHD) is the main com-
plation after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Peroxisome proliferator-activated receptor (PPAR) 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:** Two hundred and fifty patients underwent allo-HSCT, of which included 13 ISD and 12 MUD. Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARγ mRNA in healthy controls was significantly lower than that in patients after allo-HSCT within 90 days (P<0.05). The expression of PPARγ mRNA holds 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 3 to 4) was lower than 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 (P<0.05). The expression of T-bet in patients with grade III-IV aGVHD was significantly lower than grade I-II aGVHD (P<0.05).

**Summary/Conclusions:** High 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.

**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 evaluate clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

**Methods:** A total of 80 patients underwent HSCT from haploidentical donors, of which included 13 ISD and 12 MUD.

**Results:** Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARγ mRNA in healthy controls was significantly lower than that in patients after allo-HSCT within 90 days (P<0.05). The expression of PPARγ mRNA holds 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 3 to 4) was lower than 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 (P<0.05). The expression 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.

**E1522**

**OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA HARBOURING INV(3)(q21;q26.2)/t(3;3)(q21;q26) IN RELATION TO THE CLINICAL OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA:**

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**Background:** Acute myeloid leukemia (AML) with inv(3)(q21;q26.2)/t(3;3)(q21;q26) is categorized as AML with recurrent genetic abnormality in the WHO classification, accounts for approximately 1%-2% of 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) remain unclear.

**Aims:** We retrospectively examined the impact of inv(3)(q21;q26) 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), who were aged ≥16 years and underwent their first transplantation between January 2000 and December 2014. We analyzed outcomes as overall survival (OS), relapse, and nonrelapse mortality (NRM) for the patients undergoing 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, age, disease status at HSCT, Hemoglobin level at HSCT, and primary site of the diseases. All data were analyzed using statistical software (SPSS, version 22). The following variables were 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 15052 patients with AML who were aged ≥16 years and who underwent their first transplantation, inv(3)(q21;q26.2)(3)(q31;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, 16.4% to 44.2%), 5.8% (95% CI, 0.4% to 38.4%), and 21.1% (95% CI, 11.8% to 35.2%), respectively. Multivariate analysis revealed that an age of ≥50 years (HR, 2.05; 95% CI, 1.06-3.99; P = 0.03) was a significant risk factor for poor OS. Non-CR at transplantation, (HR, 2.55; 95% CI, 0.94-6.93; P = 0.07), and reduced conditioning intensity

**Figure 1.**

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 (MUD) 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.

Figure 1. 22nd Congress of the European Hematology Association
(HR, 2.03; 95% CI, 0.99-4.14; P=0.05) were risk factors with marginal significance for poor OS.

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 (Nath CE Br J Clin Pharmacol. 2010 May; 69(5):484). PG-free MEL (EvomelaTM) 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 myeloblation 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>252000. 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 are 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 MO2 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 X10^3/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 x10^3/µL, range: 1 to 3.1) and low (n=51, median=0.9 x10^3/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+ sub-population 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=.07) and OS (P=.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.

Figure 1.

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 patients who received high-dose intravenous cell-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 regimens as conditioning with 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, which 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 bone marrow 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 pre-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 (rs2856841), IL2 (rs2069762), IL4 (rs2245250), IL6 (rs1800795), IL10 (rs1800871), IL17A (rs2275913), CD14 (rs34424920), TNFα (rs1800629), FCGR2A (rs1801274) was performed by polymerase chain reaction with allele-specific primers (Lithe, 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 leucocytes ≥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: 0.60-283, 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 autologous HSCT. Identification of the wild-type allele in intron gene IL17A (G-197A) 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 NEUTROPIA**

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1Laboratory of Immunology of Leukemia, 2Department of chemotherapy and blood transfusion were prolonged febrile neutropenia episode. 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](image-url)

**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 antibacterial prophylaxis. Four patients receiving granulocyte transfusion, on the 13th-18th days of transplantation, their neutrophil counts were 0.03-0.08x10^3/dl.
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 days-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, Karnofsky 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 the majority of non-relapse deaths (n=15). Of the patients alive, only 11 (8%) had chronic renal impairment. Chronic GvHD was associated with these patients (73%) one of whom was dialysis dependent.

Summary/Conclusions: AKI is 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 conferred 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.

E1530
PREDICTIVE INDEXES FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION, A SINGLE-CENTER EXPERIENCE
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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is often associated with complications such as graft-versus-host disease (GVHD), resulting in poor outcome, relapse and death. Introduction of reduced intensity conditioning (RIC) regimens and improvements in supportive care, have allowed offering allo-HSCT to more and older patients (pts). A balanced risk-benefit approach of candidates for allo-HSCT is the key for maximized chances of cure with acceptable quality of life.

Aims: Compare the potential utility of two pretransplant predictive models: PAM (pretransplant assessment of mortality, Parinom et al, AIM 2006) and HCT-CI (HCT comorbidity index; Sorror et al, Blood 2005), in our cohort of pts.

Methods: We retrospectively studied 154 pts, 86 (55.8%) were males with a median age of 51 years (range: 15-68), who underwent allo-HSCT in our center between May 2005 and December 2014. Patients’ baseline diseases were: acute myeloblastic leukemia (24%), multiple myeloma (22.7%), non Hodgkin lymphoma (11.7%), acute lymphoblastic leukemia (11%), myelodysplastic syndrome (9.1%), chronic lymphocytic leukemia (5.2%), Hodgkin lymphoma (3.9%), aplastic anemia (3.9%), myelofibrosis (3.9%), chronic myeloid leukemia (1.3%), Waldenström macroglobulinemia (1.3%) and others (1.8%). Eighty (51.9%) pts received cells from matched siblings, seventy (45.5%) from unrelated donors and the remainder (4.5%) received RIC regimens. Stem cell source were: peripheral blood (n=86), bone marrow (n=63) and umbilical cord (n=5). Median and maximum follow-up were 31 and 228 months, respectively.

Results: Median age of 51 years (range: 15-68), who underwent allo-HSCT in our center between May 2005 and December 2014. Patients’ baseline diseases were: acute myeloblastic leukemia (24%), multiple myeloma (22.7%), non Hodgkin lymphoma (11.7%), acute lymphoblastic leukemia (11%), myelodysplastic syndrome (9.1%), chronic lymphocytic leukemia (5.2%), Hodgkin lymphoma (3.9%), aplastic anemia (3.9%), myelofibrosis (3.9%), chronic myeloid leukemia (1.3%), Waldenström macroglobulinemia (1.3%) and others (1.8%). Eighty (51.9%) pts received cells from matched siblings, seventy (45.5%) from unrelated donors and the remainder (4.5%) received RIC regimens. Stem cell source were: peripheral blood (n=86), bone marrow (n=63) and umbilical cord (n=5). Median and maximum follow-up were 31 and 228 months, respectively.

Figure 1.

Results: After allografting, 57.1% pts had complications, the most frequent were: infections (45.5%), followed by nephrotoxicity (25.3%), hepatotoxicity (12.3%), pulmonary toxicities (9.7%) and cardiotoxicity (3.9%). Eighty-two percent of pts with high risk/intermediate risk group of PAM score presented complications; versus 46% of pts included in low/intermediate risk (p<0.001). Regarding GVHD, 41.6% and 31.2% of pts developed aGVHD (grades II to IV) and cGVHD, respectively. PAM score was a good predictor for aGVHD risk: 38.1% of pts with low/intermediate risk had aGVHD versus 59.3% of pts with high/very high risk (p=0.001). On multivariate analysis, the risk of cGVHD (caused by non-relapse causes) in our cohort of pts was 26%. Causes of NRM included infections (45.8%), hemorrhage (10%), pulmonary toxicities (16%), second neoplasia (14.6%), GVHD (6.2%), cardiotoxicity (2%) and hepatic toxicity (2%). PAM score effectively risk-stratified pts for NRM: 17%, 24.7%, 45.8%, and 50%
in the low, intermediate, high and very high risk groups, respectively, showing a clear distinction by categories (p<0.038) (figure 1). Refraining, relapse, 44 (28.6%) pts 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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**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 allogeneic 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 53%, respectively. When considering incidence of disease relapse or progression (CIR) and nonrelapse mortality (NRM), the 5-year CIR 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), CIR (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 HEPATOCELLULAR CARCINOMA: 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:** 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 (renal, pulmonary) was permitted. After informed consent, defibrotide 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 (4.5 years; 19% of patients) or ≥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>All HSCT Patients</th>
<th>Age ≤16 Years</th>
<th>Age &gt;16 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>96</td>
<td>85.9%</td>
<td>93.0%</td>
</tr>
<tr>
<td>≥2 to &lt;3</td>
<td>535</td>
<td>55.5%</td>
<td>378</td>
</tr>
<tr>
<td>≥3 to &lt;5</td>
<td>204</td>
<td>42.7%</td>
<td>120</td>
</tr>
<tr>
<td>≥5 to &lt;8</td>
<td>39</td>
<td>53.7%</td>
<td>22</td>
</tr>
<tr>
<td>≥8</td>
<td>13</td>
<td>33.9%</td>
<td>11</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.
Background: The prognosis of patients with newly diagnosed peripheral T-cell lymphomas (PTCLs) is very poor following conventional therapy alone with 5-years PFS ranging from 20% to 30%. From 2006 to 2010, we conducted a multicenter prospective phase II trial to evaluate the efficacy of upfront consolidation of clinical response with autologous (auto) or allogeneic (allo) stem cell transplantation (SCT) in patients at diagnosis. The results were previously reported (4 year PFS of 70% and 69% for auto and allo SCT, respectively) (Corradini P. 2014).

Aims: In this analysis, we extended the follow-up of our trial.

Methods: SCT: Fifty patients were enrolled after central review pathology (Peripheral T-cell Non Hodgkin Lymphomas (PTCL-NOS) n=33, Anaplastic lymphoma kinase-negative anaplastic large cell lymphomas (ALCL) n=12, Angioimmunoblastic lymphomas (AITL) n=14, enteropathy-associated T-cell lymphomas (EATL) n=2). The induction therapy consisted of 2 courses of CHOP and alemtuzumab followed by 2 courses of high-dose methotrexate, cytarabine and cyclophosphamide. Patients in clinical remission with HLA identical donors received allo SCT whereas those without a suitable donor received auto SCT.

Three patients relapsed after auto SCT and were subsequently allografted (data of last follow-up was censored at date of second transplantation procedure).

Results: Only 37 patients underwent transplantation (autologous SCT (n=14), allogeneic SCT (n=23)) whereas 24 did not for toxicity (n=5), progressive disease (n=18) or clinical decision (n=11). In intention to treat analysis, at a median follow-up of 76 months, the estimated 7-years progression-free survival (PFS) and overall survival (OS) were 51% (95% CI 36%–66%) and 62% (95% CI 46%–83%) in patients autografted and allografted, respectively. We did not observe a significant difference in OS between auto or allo consolidation 69% (95% CI .31%–88%) versus 63% (38%–79%) (p=0.51), but 3 patients in relapse after auto SCT were allografted. The main cause of failure after auto SCT was relapse (6 of 14, last relapse occurring at 81 months after auto). The crude cumulative incidence of non-relapse mortality and relapse after allo SCT were 19% (n=4 deaths, one patient died of cardiac complication 62 months after allo SCT) and 17% (n=4 deaths, respectively).

Summary/Conclusions: The long-term outcome of patients receiving any transplantation strategy remains satisfactory. In the future, biological markers could help physician to select the better therapeutic option for the patients.

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 (BBMR) provides UDs to international (mainly European) centres (TCs) and to UK TCs via the Anthony Nolan registry. Data reported by international registries on donor attrition is scarce and mainly focused on attrition at the VT stage. BBMR 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 BBMR donors at the post-VT stage.

Methods: Data on requests for work-ups from April 2002 to December 2016 were extracted from BBMR databases and donor notes and were analysed retrospectively. The reasons for cancellation were categorised: cancellation initiated by the donor (n=78) or by the BBMR (n=37); the reasons were further divided into 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 BBMR final/backup 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 initiated 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 older than 50 years were more frequently pulled out. Donor pull-out showed a significant association 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, WMRA Annual Report 2015). This is likely due to the fact that most BBMR donors are regular blood donors: few donors withdrew for personal reasons. Medical reasons for frequent cause of cancellation for donor reasons. 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 BBMR provide reliable and accessible stem cell donors.

E1535 POLIMORPHISM IN TGFβ1 GENE PREDISPSES TO RELAPSE AND DEVELOPMENT OF ACUTE GRAFT-VERSUS-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 hematological malignancies. Cytokines play a well established role in the mechanism of acute GvHD (aGvHD), which is one of the most significant complications after allo HSCT transforming growth factor B1 (TGFβ1) is one of the inflammatory cytokines, which play a pivotal role in the development of aGvHD.

Aims: The aim of this study was 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-II: 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-II 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 C variant, but the difference did not reach 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 was 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.
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 lose 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 and 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 myeloablation 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=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 VACARIUS 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 surface inducing 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 C/T with 1 additional nucleotide insertion (O) 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 A5.1 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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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 old patients treated with the TS Bu (TS cohort) or the RIC FluBu 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 FluBu regimen, which is used as standard (RIC cohort) for older patients at our center.

Methods: Patients in the TS cohort received Flu 80mg/m2/day on day 13 and -12 and Flu 40mg/m2/d followed by IV Bu on day 6 to -3, dose adjusted to achieve a total Bu course AUC of 16,000μmol-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) dose adjusted 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 2015.

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 longer PFS with TS-MAC regimen (HR: 0.36; P=0.003). The benefit was mainly seen in patients with a comorbidity score ≥3.

Table 1.

Summary/Conclusions: The myeloablative timed sequential Bu regimen improves survival and appears promising in older patients with AML/MDS. The myeloablative timed sequential Bu regimen improves survival and appears promising in olderpatients with AML/MDS.
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) possesses 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 four 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 GvHD, 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 x10⁹/kg TcR Αβ.

Results: The mean of collected CD34 cells were 18.60 (range 3.98-43.66) x 10⁶/kg. The graft had a purity of 99.9% TCRαβ depletion with a median of 0.257 (range 0.003 to 1.47) x 10⁶ 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 and GvHD were observed in three patients. Although the patients with gastrointestinal GvHD were treated with steroid, budenoside, 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 regarding immunological reconstruction 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-year-nine 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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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 related donor programs, donor age, disease status and previous therapy lines. The availability of a haploidentical donor in most families is a potential advantage.

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 genetic 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 (=<50x10⁹/μl or <50% of normal baseline). Cases without signs of renal or pulmonary dysfunction were excluded.

Results: We identified 17 TA-TMA episodes treated with defibrotide in 16 allo genetic transplant recipients: 9 men; median age 38 years old (16-57); 10 single-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). Compliant 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 septicaemia) or bacterial (1 E Coli sepsis) infections. Median onset of TA-TMA was on day +43 after transplant (2-56), 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
Pre-transplant comorbidity as an outcome predictor in hematopoietic cell transplantation for severe aplastic anemia

S.-N. Lim1,*

Pre-transplant comorbidity as an outcome predictor in hematopoietic cell transplantation for severe aplastic anemia. 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 autologous 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 BMTR 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 infusions, 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 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, 4-1178.4 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 63.8%, 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 84.1%, 68.6%, and 60.6%, respectively (P=0.007). The EFS for HCT-CI 0, 1-2, and ≥3 at 4 years was 76.5%, 60.0%, and 56.3%, respectively (P=0.019). 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).

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

Efficacy 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 (PBSC) 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 PBSC mobilization for harvesting and hematopoietic SCT.

Methods: Retrospective, controlled, observational study conducted at the University Hospital of Salamanca between January 2008 and December 2015.

Results: The study included 365 patients candidates for ASCT and 217 healthy sibling donors for Allo-SCT who underwent PBSCs mobilization. Neupogen® (Amgen Europe BV, Breda, NL) was administered for mobilization at standard doses until SEP2012, while Nivestim® (Hospira, Maidenhead, UK) was used from then on. Among PBSCs, 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® 732±133; Nivestim® 732±133, SD=113.9; Nivestim® 65.3±64.5, SD=66.0; p=0.15), but the mean of the total CD34+ collected cells was 4.75. SD=4.4 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=6.5 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% 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 7.62x10^6, SD=3.45x10^6 for Nivestim® vs 6.26x10^6, SD=2.71x10^6 Neupogen® (p=0.002), but the minimal target cell dose (2x10^6/kg) was collected in all donors. 8.5% of donors mobilized with Nivestim® failed to achieve the optimal cell dose (4x10^6/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.**

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<th>Characteristics and main results comparison in patients who underwent autologous stem cell transplantation</th>
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**Figure 1.**

**E1545**

LONG-TERM RESULTS OF DONOR LYMPHOCYTE INFUSIONS IN RELAPSED AND MIXED CHIMERISM PATIENTS AFTER ALLOGENEIC STEM CELLS TRANSPLANTATION

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**Background:** Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for patients with hematological malignancies. However, relapse remains the major cause of treatment failure after allo-HSCT. Mixed chimerism (MC) can induce immunologic tolerance and lead to relapse. One of the most effective approaches to treat these patients is donor lymphocyte infusion (DLI) with or without chemotherapy.

**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 (18-54 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 1x10^7CD 3+ per kg. Every following dose of infusion CD 3+ increased until 5x10^7CD3 + 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 causes. 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 (18-60 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 occurred 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. Disease free survival 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 hematological 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 naïve cells (CD45RA+), predominantly cytotoxic Tlymphocytes, 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-leukemia and anti-rejection properties.

**Aims:** We describe the outcome of CD45RA+ cell depletion of donor lymphocytes infusions, in patients with relapsed 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 6.4x10^6/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 8-18 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 counts of blast cells CD45RA+ in haploidentical donor lymphocytes, the CD45RA+ cells depletions was made using the clinMACS system. The median dose of CD45RO+ cells was 1.02x10^10/kg, starting at a dose of 3x10^10/kg on day +1 to a maximal dose of 2x10^10/kg within first 6 months after transplantation. After transplantation, 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±7.8%. In the primary multivariate analysis (including age, cycles of pre-transplantation chemotherapy, bone marrow blasts, cytogenetics and treatment center), only bone marrow blast ≥35% and age over 40 were associated with disease-free survival and relapse respectively while there was no signific-ant difference between RJJH 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-Bus 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. Stutter PCR products may produce false-negative or false-positive chimerism estimation at the point of low recipient hemopoiesis 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.

**Figure 1.** 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. Stutter PCR products may produce false-negative or false-positive chimerism estimation at the point of low recipient hemopoiesis output.

**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 the 210 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 v.4-0 software. Informative loci were chosen beforehand comparing pretransplant
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 hemogamy; 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 13.6% 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);(1-stuffer/total DNA ratio)*100% (special formula for hetero- and homozygous on fig.1). To test these formulae the panel of DNA samples with mixed chimerism from 50 to 97% estimated by independent "stuffer-free" markers.

The results of chimerism estimation based on "stuffer-complicated" markers (using proposed formulae) conventional "stuffer-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.

E1550

PERIPHERAL BLOOD STEM CELL (PBSC) HAPLOIDENTICAL TRANSPLANTATION VERSUS MISMATCHED UNRELATED DONOR TRANSPLANTATION: A SINGLE UK CENTER STUDY

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Background: Haploidentical (Haplo) and mismatched unrelated donor transplantations (MMUD) are novel and attractive alternatives for allotherapy in patients with a fully matched available donor. 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 v2.30 and R 3.3.2 statistical software.

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 leukemia (AML) was the commonest transplant indication in both groups (60.5% of Haplo and 93.6% of MMUD transplants). The disease risk index (DRI) in this subgroup was overall low/intermediate in 69.2% and high very high in 26.2% (unknown in 4.6%). Reduced intensity conditioning was used in all but two Haplo (6.4%) and 4 MMUD transplants. Patients were followed up for a median of 544 days with a similar 2-year overall survival of 61.6% (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. Median time to neutrophil engraftment was 18 and 12 days in Haplo and MMUD transplants respectively. 25.5% (95% CI 12-41%) and 31.2% (95% CI 18-45%) respectively; p=0.61 and 34.8% (95% CI 20-49%) respectively, p=0.51. Median time to neutrophil engraftment was 18 and 12 days and for platelet engraftment 21 and 12 days in the Haplo and MMUD transplants respectively. Engraftment was successful in 89.4% (Haplo) and 95.5% (MMUD) of patients. The incidence of acute GVHD was similar in Haplo and 35% in MMUD transplants but severe grade 3/4 acute GvHD occurred in 7.9% (Haplo) and 8.9% (MMUD). Chronic GvHD occurred in 15.8% of Haplo and 33.3% of MMUD transplants, p=0.067. Chronic GvHD did not impact overall or progression free survival in either transplant group.

Conclusions: Haploidentical hematopoietic 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.
LOW BLOOD CONCENTRATION OF TACROLIMUS CAN BE A RISK OF GRAFT FAILURE AFTER BLOOD STEM CELL TRANSPLANTATION

A. Fujimoto1,*, T. Ishikawa1

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 recovery, Tac blood level (median: 15ng/ml), Tac dose (mean: 12.5mg/kg), and Tac serum level (mean: 15ng/ml) in 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 mainlyAML (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 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<0.001). 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 ABOi vs 24.69 in ABOc; 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

E1553

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 an unexplored field.

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.

E1552

LOW BLOOD CONCENTRATION OF TACROLIMUS CAN BE A RISK OF GRAFT FAILURE AFTER BLOOD STEM CELL TRANSPLANTATION

A. Fujimoto1,*, T. Ishikawa1

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 an unexplored field.

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.

Figure 1. Summary/Conclusions: Low levels of Tac blood concentration were significantly associated with the incidence of graft failure of the patient for whom Tac with an additional sMTX were used for GVHD prophylaxis. Before engraftment, frequent checks of the Tac blood concentration and maintaining the drug level should be considered for these patients.
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 megachemotherapy with autologous stem cell transplantation and at time of hematopoietic recovery in patients after PBSCT. Relative expression of Toll-like receptors was assessed by real-time PCR using inventoried TaqMan® Assays from Life Technologies/Thermo Fisher. Beta glucorondidase (GUSB) served as endogenous control. Reaction was performed in 7500 Real Time PCR instrument (LifeTechnologies) using Gene Expression MasterMix (LifeTechnologies/Thermo Fisher). Comparative C 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 histograms of Lilliefors 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 qualitative 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 at the level of p<0,05 were assumed to be of statistical significance. Results: The mRNA expression of TLR2 and TLR9 was significant higher in patients after PBSCT than before PBSCT procedure (ΔCt TLR2 1,4290±1,0461 vs 1,7877±1,4974 and ΔCt TLR9 117,834±1,0870 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 117,834±1,0870 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 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 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 25 mg/kg/d for 14 days, or to a maximum of 28 days, or until HSCT recovery, or until patient withdrew consent. Disease progression was assessed by changes in laboratory tests and imaging. If HSCT patients were alive and >30 days post-HSCT and had received >28 days of defibrotide, a post hoc analysis was performed to evaluate the impact of timing of defibrotide on survival. For both primary events and adverse events, Fisher's exact test and chi-square tests were used to assess the impact of defibrotide on VOD/SOS and MOD.

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 and 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<0.01), 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<0.01). 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%).

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<0.01). 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.
Aims: RDW values were evaluated at the day of infusion (RDW 0), we choose 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 GHVD. 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 haploidential 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’s is an easy and affordable prognosis marker for sGVHD that should be further evaluated.

E1556
COMPARISON OF THE BEAM CONDITIONING REGIMEN AND THE BEAM CONDITIONING REGIMEN IN THE AUTOLOGOUS TRANSPLANATATION 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 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 the National Specialized Hospital for Active Treatment of Hematological Diseases in Sofia for relapsed/refractory HL or NHL for the period from 1.01.2013 to 1.07.2016 with a follow-up of patients up to 1.11.2016. 92 of the patients received BEAM and 22 received BeEAM. 2 and 3 year OS and DFS were compared, CR rates and the average time periods to hematological recovery.

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% for BEAM, provided that the differences did not have statistical significance. The CR rate was 63.63% in the BeEAM group versus 50% in the BNCU 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.

Figure 1.

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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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 centers 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 8 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 ≥4/6 match at A, B (serologically) and DRB1 (by HRT). Combined TNC count for the units had to be ≥3×10^10/kg recipient weighting. Condition was Fludarabine 40mg/m2 x4 and TBI 150 cGy x8; 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>0.5×10^9/L at median of 20 days (range 14-72). Platelet count reached >20×10^9/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-
uus 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%) had 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 3-4 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>
<thead>
<tr>
<th>HLA Match</th>
<th>Higher TNC</th>
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<th>Lower TNC</th>
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<td>Better (&gt;3)</td>
<td>34(3%)</td>
<td>4(21%)</td>
<td>27(14%)</td>
</tr>
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<td>Same (1-2)</td>
<td>29(53%)</td>
<td>27(53%)</td>
<td>8(14%)</td>
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<tr>
<td>Worse (&lt;1)</td>
<td>35(64%)</td>
<td>25(49%)</td>
<td>13(24%)</td>
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E1558

CLINICAL ANALYSIS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR 46 ACTIVE RELAPSED AND REFRACTORY ACUTE PEDiATRIC LEukEMA

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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) for children with AML. HSCT was offered 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 who underwent HSCT in our institution from 2012 and 2016. Median age at HSCT was 13 years. Active R/R disease was all confirmed by cytogenetics/molecular genetics and aggressive clinic 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. Varied post-HSCT intervention including donor lymphocytes infusion and intralumino-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 patients died before 90 days due to relapse. Transplant-related mortality at one year 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 25%. Therapeutic recommendations for pediatric subjects with a similar situation is not available.

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. Within adult HLA disparity 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.
Results: The patients were 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 from relapse was 10, 20 and 25 days in the PSC, CHT and IT groups, respectively. In the CHT group, 3 patients (16%) achieved a CR and 4 (22%) had PR during induction 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 46 patients, median overall survival (OS) was 7 months (range 2-74), being 4, 5, 3 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 support the need to schedule a prospective project combining cytokerotic treatments and immunotherapy in patients in AML relapsing after allo-SCT.

E1562
RESULTS OF THE IMPLEMENTATION OF CRYOTHERAPY IN PROTO-
COYS OF ORAL MUCOSITIS PROPHYLAXIS IN PATIENTS SUBJECT TO A TRASPLANT OF HEMATOPOYETIC PROGENITORS. EXPERIENCE OF ONE CENTER

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Background: Oral mucositis (OM) is one of the main complications 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 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 hemodialysis regimen in which cryotherapy was not applied (2007-2011) and another cohort in which was applied (2011-2015) where cryotherapy was being 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 multivariate and univariate 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 (54% vs 72%, p=0.149). The use of parenteral nutrition was lower in the non-cryotherapy group (8.5% vs 15%, 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=0.04). The number of days of 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). Hazz ratio was 0.81 (IC 95% 0.06-0.55).

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 cryother-

Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NON-CRYOTHERAPY</th>
<th>CRYOTHERAPY</th>
<th>p</th>
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<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>55%</td>
<td>54%</td>
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<tr>
<td>Age Median</td>
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<td>54</td>
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<tr>
<td>CR at 100 days</td>
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<td>GVHD grade</td>
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<tr>
<td>MDS</td>
<td>3%</td>
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E1563
REDUCED INCIDENCE OF PRIMARY GRAFT FAILURE IN PATIENTS UNDERGOING HAPLOIDENTICAL STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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1Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Spain
2Hospital Universitario de La Princesa, Madrid, Spain

Background: Reduced intensity conditioning, 59% received matched sibling donor and 41% matched-

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 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 hemodialysis regimen in which cryotherapy was not applied (2007-2011) and another cohort in which was applied (2011-2015) where cryotherapy was being 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 multivariate and univariate analysis. Hazard ratios and 95% were estimated with the use of logistic regression model.

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Decreased mucositis degree was associated in both univariate and multivariate analysis only with cryotherapy (p = 0.01 and p=0.0003). Hazz ratio was 0.81 (IC 95% 0.06-0.55).

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Aims: The use of haploidentical hematopoietic stem cell transplantation (HSCT) as an alternative to HLA-matched allo-HSCT is increasing due to the shortage of HLA-matched donors. The aim of this study is to compare outcomes of patients who underwent haploidentical HSCT to those of HLA-matched HSCT in terms of donor, source, graft engineering or conditioning.

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/TCRab depletion, n= 6; and 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 tier 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 series. 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.

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Disease</th>
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<tbody>
<tr>
<td>17-22</td>
<td>M</td>
<td>Acute Lymphoblastic Leukemia (ALL)</td>
</tr>
<tr>
<td>23-30</td>
<td>F</td>
<td>Chronic Myeloid Leukemia (CML)</td>
</tr>
<tr>
<td>31-60</td>
<td>M</td>
<td>Multiple Myeloma (MM)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>F</td>
<td>Myelodysplastic Syndrome (MDS)</td>
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</table>

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 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

B. Aguado1, E. Jimenez Barral1, A. Arriero1, J. Cornago1, J. Vázquez1, I. Vicuña1, V. Gomez1, R. de la Camara1, A. Figueras1, A. Alegre1
1Hematology, H.U. La Princesa, Madrid, Spain

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 granulocyctic 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. Donors were asked to sign informed consent after a full description of the procedure. PBPC were collected for autologous transplantation. The main aim was to study donors characteristics and PBPC yields in patients with lymphoma.

Results: Among the 189 donors, 85 were females and 104 were males (45% vs 55%). The hematopoietic pathologies that motivated transplantation were: Hodgkin’s Lymphoma (28%), Non-Hodgkin’s Lymphoma (47%), Myelodysplastic Syndrome (MDS) (13.8%), Acute Lymphoblastic Leukemia (ALL) (10.1%), 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 11.8%. A total of 158 donors were younger than 70 years of age and 31 donors were older than 70 years of age. Median yield of CD34+ per kilogram collected was 3.55 x 10^6 in patients older than 70 years of age and 5 x 10^6 in patients younger than 70 years of age. Median of CD34+ collected per kilogram was 4.5% of all patients in that age range. The median yield of CD34+ per kilogram collected was 8 under 70 years of age and 65% of all donors over 70 years of age. Donors older than 100 kg, median of processed volume was 18 liters. Two aphereses 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+ per kilogram collected was 5 x 10^6. Among the age ranges, median yield of CD34+ per kilogram collected was 3.55 x 10^6, in patients between 31 and 69 years was 4.96 x 10^6 and in patients younger than 30 years was 5.5 x 10^6. 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 donors 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
ALLORESPONSES OF HUMAN T-CELLS FROM ADULT PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD ARE DIFFERENTIALLY IMPACTED BY LENALIDOMIDE - IMPLICATIONS FOR AHSCT

C. Besley1, E. Kotsiou1, R. Petty1, A. Sangaralingam2, C. Chelala2, E. Ghazaly1, R. LeDieu1, J. Gribben1, J. Davies1
1Centre for Haemato-Oncology, 2Centre for Tumour Biology, Barts Cancer Institute, London, United Kingdom

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 (AHSCT). 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.

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 allostimulation 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.

Methods: We 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 (GMPB), 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 succinimidy l 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.
Aims: To identify individual patients at risk before the onset of aGvHD.

Methods: To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated wild type controls.

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.

E1569

SURFACE RECEPTOR EXPRESSION PROFILE DEFINES ALLOREACTIVE DONOR CD8+ T-CELLS AFTER MURINE ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION

C. Tjia1, M. de Visser1, M. Ourens1, T. C. Brede1, A.-L. Jordán Garrote1, S. S. Riedel1, M. Chopra1, A. Mottock1, S. Thusek1, M. Ritz1, K. Mattenheimer1, C. Graf1, H. Einsele1, P. G. Schlegel1, R. S. Negrin1, A. Beilhack1

1Medical Department II, University Clinics Wuerzburg, Wuerzburg, 2Wuerzburg University, Institute of Pathology, 3University Clinics Würzburg, Department of Pediatrics, Würzburg, Germany, 4Division of Bone & Marrow Transplantation, Department of Medicine, Stanford University, United States

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, Thy1.1+) 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 CD45R (integrin, and P- and E-selectin ligand highly up-regulated on alloreactive donor T 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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1Biochemistry laboratory, 2Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

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: 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 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, at the same time, the possibility of treating developing diseases by prescribing medication makes the problem highly relevant.

Results: We have found the features of impaired balance in OS-AOS in MM patients before as well as in course of auto-HSCT.

Summary/Conclusions: Further, we have used PBMNC and benzene as potential predictive markers.

E1567

E1569
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 MAJOR
P. Ricchi1,*, A. Meloni2, S. Costantini1, A. Spassiano1, T. Di Matola3, A. Pepe2, L. Pistola1, P. Cinque1, A. Filosa1
1AORN A. Cardarelli, Naples, 2AORN Monaldi-Cotugno-CTO, Naples, Italy
22nd Congress of the European Hematology Association

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 Hbb, 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 Thalassemia (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.

Summary/Conclusions: Low serum ferritin values, even in the normal range, do not per se exclude cardiac and hepatic iron overload, although decreasing the risk. Before to consider a reduction of the chelator dose in patients whose serum ferritin levels have reached the target, a MRI scan should be performed in order to measure iron levels in the different organs.

E1571
LOW SERUM FERRITIN LEVELS DO NOT PROTECT FROM CARDIAC AND HEPATIC IRON IN PATIENTS WITH THALASSEMAIA MAJOR
A. Meloni1,*, A. Spassiano2, P. Ricchi2, L. Pistola1, A. Carrà3, C. Cosmi1, R. Rossò2, A. Scaccetti6, V. Positano1, R. Righi7, S. Renne8, A. Pepe1
1AORN A. Cardarelli, Naples, 2AORN Monaldi-Cotugno-CTO, Naples, Italy
3Radiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

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 intimamedia thickness (CIMT) 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, 82% specificity, 87% positive predictive value and 82% negative predictive value.

Summary/Conclusions: Low serum ferritin values, even in the normal range, do not per se exclude cardiac and hepatic iron overload, although decreasing the risk. Before to consider a reduction of the chelator dose in patients whose serum ferritin levels have reached the target, a MRI scan should be performed in order to measure iron levels in the different organs.
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 IMAT 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 IMAT 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). IMMA and MDA levels were positively correlated (r=0.503, p=0.001) and there was a significant positive correlation between these two markers and serum ferritin (IMAT: r=0.545, p<0.001 and MDA: r=0.567, p<0.01) among TM patients. IMMA levels were positively correlated to TRV (r=0.621, p=0.008) whereas negatively correlated to ejection fraction (r=0.412, p=0.014) and fractional shortening. Both IMMA and MDA were positively correlated to CIMT (r=0.607, p<0.001 and r=0.635, p<0.001, respectively).

Summary/Conclusions: Our results highlight the role of oxidative stress in the pathophysiology of vascular complications in thalassemia. IMMA 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 NARIURETIC PEPTIDE LEVEL AND ECHOCARDIOGRAPHIC TISSUE DOPPLER ABNORMALITIES IN PATIENTS WITH BETA 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) and 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 immunosorbanct 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 and non-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 0.55±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 212.31±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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1Thalassemia Unit, 2Obstetric Department, Hippokration General Hospital, Thessaloniki, 3Thalassemia Centre, Laikon General Hospital, Athens, 4Microbiology Department, 5Obstetric Department, Aristotelion University, Thessaloniki, Greece

Background: Hemoglobinopathies constitute the most frequent monogenic disorders worldwide and thalassemias are the most frequent genetic disorders among European populations. The frequency of hemoglobinopathies is 2.5% 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 as couples or as single individuals of thalassemia prevention. 3.659 couples were 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 at 12 weeks of gestation (n=298), in few cases by amniotic fluid sampling (n=21) collected at 16-18 weeks. Few late comers 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, Hbe/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-thalilent β-thal. 91% of the couples were of Greek origin, and 9% were immigrants from Africa, Asia, Middle East, South America, India, Pakistan, Nigeria, Pakistan, in Latin America, and Asia.

Results: We analyzed the impact of the presence of fatty liver in the parameters of iron overload among our patients with Non Transfusion dependent Thalassemia (NTDT).

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 (NTDT).

Methods: 111 patients with NTDT were cross-sectionally evaluated; the diagnosis of liver steatosis was ultrasound-based (US). In all patients ferritin levels and serum alanine aminotransferase (ALT) to serum aspartate aminotransferase (AST) ratio were assessed. Liver iron concentration (LIC) measurements were available for 64 patients (54%) who underwent a magnetic resonance Imaging (MRI) scan within the Myocardial Iron Overload in Thalassaemia (MIOT) network.

Results: Liver steatosis was frequently (35.5%) encountered among our patients with NTDT 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 ratio >0.89 predicted the presence of liver steatosis with a specificity=0.872 and a sensitivity=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
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 INEFFECTIVE ERYTHROPOIESIS IN BETA-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 related to clinical severity, including cancer, chronic inflammation, autoimmune diseases and trauma. The mechanisms of release of cfDNA 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 cfDNA is mainly unclear. It has been suggested that cfDNA, at least after bone marrow transplantation, could be mostly of hematopoietic origin. This finding prompted us to explore whether cfDNA is increased in patients with ineffective erythropoiesis (IE), a condition mostly of hematopoietic origin. The aim of our study was to prospectively assess whether the Piga’s positive 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-compacted 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.

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=1.8-11.36, 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). Based on our data a NC/C ratio >2.5 provides prognostic information for patients with TI about the risk of developing cardiac complications.

Figure 1.

E1577
LEFT VENTRICULAR HYPERTROBECULATION BY CARDIAC MAGNETIC RESONANCE IN THALASSEMIA INTERMEDIA PATIENTS: FREQUENCY AND PROGNOSTIC ROLE
A. Meloni1, F. Macaroni2, L. Pistoia1, S. Pulini3, V. Santamarina4, M. Benni5, L. Sardella6, A. Barison1, G. Poritore5, V. Postiano1, S. Novo2, A. Pepe1

Background: Differences of left ventricle non-compaction (LVCN) from hypertrobeculated 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 LVCN 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-compacted 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.

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=1.8-11.36, 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). Based on our data a NC/C ratio >2.5 provides prognostic information for patients with TI about the risk of developing cardiac complications.
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 dependentthalassemias and in sickle cell disease, but yet not 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 form 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 (TRV>2.5m/sec.) underwent cardiac catheterization.

**Results:** The present study included 52 thalassemic patients, 28 males and 24 females. Age ranged 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 THALASSEMA MAJOR PATIENTS.**

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**Background:** The new parameters of cardiac function, derived from two-dimen-
sional speckle-tracking 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-tha-
assemia patients aged between 11–20 years recruited from the Pediatric Hema-
tology and Oncology 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 Track-
ing),MRI T2* were done.Cardiac affection by speckled was defined as decreased longitudinal strain less than 11 percentage or affection of any seg-
ment 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 short-
ening fraction, while 2 had iron overload by Cardiac MRI T2*. Patients with mean serum ferritin >3000 ng/mL in the last 2 years prior evaluation showed a signifi-
cantly lower longitudinal strain (GLSPLAX) (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 speleconornized and non splenc-
tomized patients as regard speckled echocardiographic measures. The ROC curve analysis revealed that GLPS AUC 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 GLSPAX &GLPSAC 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* techn-
ique was the reference standard for 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 THALASSEMA: A SYSTEMATIC REVIEW**

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**Background:** Pharmacological agents such as hydroxyurea promote fetal hemoglobin production via a reactivation of γ-genes. In β-thalassemia there is an imbalance in globin chains which could be ameliorated by the newly syn-
thesized 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-thala-
ssemia 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 inde-
pendent 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>
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</thead>
<tbody>
<tr>
<td>β-thalassemia major</td>
<td>35 (52%)</td>
<td>35 (52%)</td>
<td>14 (21%)</td>
</tr>
<tr>
<td>β-thalassemia intermedia</td>
<td>600 (87%)</td>
<td>600 (87%)</td>
<td>14 (21%)</td>
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</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 hydroxy-
urea therapy on beta thalassemia major patients, (n=11, 36%) evaluated beta thalassemia intermedia patients while (n=10, 34%) included both beta thalas-
assemia 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 (8-15mg/kg) Table I showing number and percentage of patients having major, partial and poor response to hydrox-
yurea 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=9, 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 MONITORING METHOD FOR DETECTION OF ALTERATIONS IN GLUCOSE HOMEOSTASIS IN BETA-THALASSEMA PATIENTS**

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**Background:** Glucose metabolism disturbances, among other endocrinopathies, are a common feature of β-thalassemia major (β-TM). Pan-
creatic 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 β-TM patients with calculation of HOMA-IR and assessment of HbA1c.

**Methods:** Thirty eligible studies comprising of a total of 1822 patients with beta thalassemia were identified. Of these (n=9, 30%) evaluated the effect of hydroxy-
urea 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 (8-15mg/kg) Table I showing number and percentage of patients having major, partial and poor response to hydrox-
yurea 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=9, 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.
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 of diabetic range (6.5-9.9%) while 5 of the 7 patients with impaired glucose tolerance had HbA1c readings in the prediabetic range (5.5-6.1%). Serum ferritin was significantly higher among patients with RBG≥ 140mg/dL (p=0.001). It was noted that 6% of patients with RBG≥ 140mg/dL were not compliable and 75% of patients on desferrioxamine therapy had RBG>140mg/dL. There was a significant positive correlation between HbA1C% and FBG among the studied thalassemia patients with elevated RBG≥ 140mg/dL, while HbA1C% was negatively correlated with fasting C-peptide. Serum ferritin was positively correlated with FBG. As regards the CGMS data, HbA1c was positively correlated to maximum blood glucose, average blood glucose, SDS blood glucose and area under the curve≥140mg/dL. The only significant independent factor for elevated RBG ≥140mg/dL was serum ferritin.

**Summary/Conclusions:** The use of CGMS in the diagnosis of early glycemic abnormalities (pre-diabetes) among patients with β-TM appears to be promising and superior to other known diagnostic modalities namely OGTT and HbA1c.

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**E1584**

**THE IMPORTANCE OF SERUM GDF-15 LEVELS TO ASSESS IRON OVERLOAD IN PATIENTS WITH THALASSEMA MAJOR**

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**Background:** Early detection of myocardial dysfunction is essential for the management of patients with thalassemia. Four-dimensional echocardiographic imaging technique that analyzes the motion of tissues in the heart may be useful for detecting subclinical cardiovascular disease.

**Aims:** To evaluate the 4-dimensional echocardiographic strain in children with beta thalassemia major and correlate it with other echocardiographic parameters.

**Methods:** This is a cross sectional cohort Study included 200 children, 1-18 years-old. They were divided into: One hundred children with β-Thalassemia major with no clinical cardiac manifestations and 100 healthy children as a control group. They were subjected to the following investigations: Complete blood count, serum ferritin and Four-dimensional echocardiographic strains (Longitudinal, Circumferential, Radial and Area strains).

**Results:** There was no significant difference between the two groups as regard mitral annulus systolic velocity (S wave), E/A ratio and iso-volumic acceleration but there was a significant difference as regard to ejection fraction, left ventricle mass, sphericity index and myocardial performance index. The mean values of Left ventricular Strains (Longitudinal, Circumferential, Radial and Area strains) were significantly lower in patients with thalassemia (-14.86±12.131, -8.01±3.829, 33.13±10.613, -19.45±6.866) than controls (-19.13±1.502, -16.32±1.34, 37.28±4.209, -22.94±3.064) respectively with a positive correlation with 2-Dimensional strain.

**Summary/Conclusions:** Strain parameters of the left ventricle obtained by four-dimensional echocardiography can be a novel and promising technique for early detection of left ventricular dysfunction in children with thalassemia.

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**E1585**

**ASSOCIATION OF SP1 POLYMORPHISM IN THE COLLAGEN TYPE I ALPHA 1 (COL1A1) GENE WITH OSTEOPOROSIS IN CHILDREN WITH BETA-THALASSEMA**

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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, Hep electrophoresis, Calcium level Serum ,alkalin phosphatase, Bone Density by DXA, Serum osteocalcin 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 genotyype there was high percentage of heterozygous Ss (G/T) and homozygous ss type there was high percentage of heterozygous Ss (G/T) and homozygous ss type respectively.

**Summary/Conclusions:** SP1 polymorphism in collagen gene could be of clinical value in identifying the thalassemic patients at risk of developing osteoporosis.
E1587

Abstract withdrawn.

E1588

VALUE OF HBA2 IN THE DIAGNOSIS OF BETA-THALASSEMA 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, due to a reduced rate of α-globin chain synthesis. The severity of the pathology is variable ranging from a very mild microcytic hypochromic anemia to a moderately severe anemia associated with a myelodysplastic syndrome.

Aims: The main objectives of this work were to characterize the molecular lesions underlying ten Portuguese cases of unusual α-thalassemia/HbH disease and to understand their origin and functional consequences.

Methods: After exclusion the most frequent molecular lesions associated with α-thalassemia, Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA P140B HBA kit (MCR-Holland) was used to search for DNA deletions in the subtelomeric region of chromosome 16p. Additionally, specifically designed synthetic MLPA probes, as well as gap-PCR and Sanger sequencing were performed for more accurate deletion breakpoint mapping.

Results: We have found five distinct deletions and one indel, all in heterozygosity. The deletions range from approximately 3.3 to 3.23 kb and two of them are novel. The three larger deletions remove the entire α-globin cluster whereas the others remove totally or partially the distal regulatory elements keeping the α-globin genes structurally intact. The indel comprises the deletion of the MCS-R2 regulatory element and the insertion of a singular 39 bp DNA fragment possibly originating from a complex rearrangement involving chromosome 3. Finally, no α-globin gene cluster deletion or point mutation were found in a patient who has been diagnosed to be very unusual case of acquired alpha-thalassemia associated with a myelodysplastic syndrome.

Summary/Conclusions: Our study widens the spectrum of molecular lesions and unusual molecular mechanisms by which α-thalassemia/HbH may occur and emphasizes the importance of diagnosing large α0-deletions to provide patients with appropriate genetic counseling.
Thrombosis and vascular biology

E1590

RELEVANT ROLE OF VON WILLEBRAND FACTOR-ADAMTS13 AXIS IN HEPATIC ISCHEMIA-REPERFUSION INJURY

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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 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 (ALF21, Advance Co, Tokyo, Japan). The hepatic blood flow was measured again 24 h after reperfusion and mice were then sacrificed for blood collection and histological analysis of liver tissue.

Results: As compared to WT mice, restoration of hepatic blood flow was significantly greater in VWF-KO mice at 24 h after reperfusion (WT: 61±17% vs KO: 87±17%, expressed as the percentage of pre-ischemic value). Consistent with the hepatic blood flow, the time-course analysis of serum alanine aminotransferase (ALT) at several time points after reperfusion revealed the lesser liver damages of KO mice (WT, 6898±3270 and 1313±621 IU/L vs KO; 3043±1320 and 478±330 IU/L, at 3 h and 24 h after reperfusion, respectively). In addition, histological analysis confirmed that neutrophil infiltration in the liver tissue of KO mice was significantly reduced as compared to WT mice at 24 h after reperfusion. These impaired hepatic blood flow and ALT values as well as intensified neutrophil infiltration in WT mice were significantly improved to an extent comparable to those of KO mice by the bolus injection of recombinant human ADAMTS13 (3 µg/mouse equivalent to 2800 U/kg, n=12) just prior to the I/R operation.

Summary/Conclusions: Our results altogether indicate that VWF-dependent inflammatory responses with neutrophil recruitment at ischemic sites are involved in pathophysiology of hepatic I/R injury, and functional regulation of VWF by ADAMTS13 may serve as a promising therapeutic option for hepatic I/R injury.

E1591

THE IMPORTANCE OF THE FULL BLOOD COUNT, JAK II AND ADAMTS13 TESTING IN STROKE EVALUATION: A REVIEW OF 619 CONSECUTIVE PATIENTS WITH Stroke and TIA

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Background: Thrombosis and vascular biology

Methods: We retrospectively reviewed full blood counts, specifically haematocrit and platelet count, and whether these were documented and further investigated if outside of the normal laboratory range. We examined whether less common primary haematological disorders known to cause stroke were considered. Adapting this for evaluation of myeloproliferative diseases such as polycythemia vera (PV) and essential thrombocythaemia (ET), and ADAMTS13 analysis for TTP

Results: 610 patients <60 years were included: 379 ischaemic stroke (62.1%), 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, 103 (16.6%) 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 leukemia. Fourteen patients had no further testing or monitoring. 26/10 (4.3%) patients had 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 life-saving plasma exchange to take place.

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. However, the 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.

E1592

PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) RELATED THROMBOSIS IN 230 PATIENTS WITH HEMATOLOGICAL MALIGNANCIES. A 6 YEARS SINGLE EXPERIENCE CENTER

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Background: The use of peripherally inserted central catheters (PICCs) is widely extended in patients with hematological malignancies, not only to be treated with chemotherapy, blood cell transfusions, but also parenteral nutrition support or frequent analytical extractions. However, catheter-related thrombosis is one of its main complications. There are a few studies that evaluate this complication. We reported the experience of the PICC-related thrombosis (PRT) in our center.

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 tunneled PICCs with different technique: 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 package 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=80; 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%), PICCs 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). PICCs 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), non-Hodgkin’s lymphoma (NHL=4), MM (4), acute myeloid leukemia (AML=4), and NHL (4). All diseased patients were tunneled (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%) PICC were 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
E1593

A STUDY OF VENOUS THROMBOEMBOLISM SUSCEPTIBILITY LOCUS FACTOR XI, ABO AND FIBRINOGEN IN A PORTUGUESE POPULATION SAMPLE
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Background: Venous thromboembolism (VTE) is a multifactorial disease caused by genetic susceptibility factors, acquired risk factors and complex 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, G6P, KN51, PROC, SLCA4A2, STXBPS, TSPAN15 and WFF, 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 (rs2066865) 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 (rs2066865) 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 Hardy-Weinberg equilibrium (HWE) 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 through 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 rs2066865 (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 HWE (p-value>0.05 for all SNPs). The logistic regression under an additive model showed that FGG rs2066865 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 after adjusting for age and sex (P=0.019 and P=0.005, respectively). ABO rs2519093 did not reach significant associations compared 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, p=0.015, OR=2.31; and 0 vs 2 or more risk alleles: χ2=12.2, p=0.0048, OR=3.36

Summary/Conclusions: Our data suggest that the alleles FGG rs2066865 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 use of central venous catheters (CVC) 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 children (85%) in the infant (0-1 years) group, 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 (NCVR) diagnoses were made in 44% of patients in 35.5% of cases due to deep extremity deep vein thrombosis (DVT) in 29% 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. Among these, 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 ANTIINFLAMMATORY DRUGS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA
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Background: Idarubicin (IDR), cytarabine (AraC), and tamibarotene (Am80) are currently used for treatment of acute myeloid leukemia (AML). In leukemia, the incidence of venous thromboembolism and disseminated intravascular coagulation is associated with induction chemotherapy.

Aims: How some drugs for the treatment of AML affect the procoagulant activity is unclear. Herein, in this study, we investigated the procoagulant effects of IDR in comparison with AraC and Am80.

Methods: Procoagulant effects of IDR, AraC, and Am80 were investigated in a vascular endothelial cell line EAHy926 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 cytometric 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, Am80 decreased TF expression and procoagulant activity, and increased TM expression on NB4 cells, we observed downregulation of TF mRNA and upregulation of TM mRNA by Am80. But Am80 did not sufficiently inhibit anticoagulant activity on NB4 cells when applied simultaneously with IDR.

Summary/Conclusions: These data suggest IDR may induce procoagulant activity in vessels by apoptosis through PS exposure and/or TF expression on vascular endothelial and AML cell lines. Am80 may suppress procoagulant changes through regulation of TF expression and induction of TM expression. Our methods could be useful to investigate changes in procoagulant activity induced by antiinflammatory drugs.

haematologica | 2017; 102(s2) | 647
Madrid, Spain, June 22 - 25, 2017
Methods: assess any trends or patterns of HAT incidence and characteristics over time. Identifying those patients with hospital-associated thrombosis (HAT), defined as complications 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 gynecology and gynaecology 4%). Not all surgeries had an index admission. 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 pre-existing medical conditions and there were no thrombotic events. Of 394 HAT cases with sufficient data, 80 (20.3%) had a preventable cause, 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 undergoing standard patient >90kg. Off those HAT cases deemed unrepreventable, 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/Conclusion: HAT rates remain stable and the majority are though clinical and by current treatments. Key errors inoviated cases are failure to perform a timely VTE risk assessment and action with appropriate thromboprophylaxis. 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, overproduction of procoagulants and deficiency/dysfunction of fibrinolyis 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) diagnosed 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 and laboratory results and analyze the association between thrombotic risk factors and thrombosis.

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 neonatal period (<1month) with a male predominancy (n=15, 68%) and from those 22 events 2 were arterial thrombosis (purpura fulminans(1), cerebral(1)) whereas 4 intracardiac, 5 sinusovenous thrombosis (deep veins(4), renal veins(3), portal veins(3) cerebral vein(1)) were noted. In 2(9%) cases of arterial 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, 5 were observed during the first month of life. Among 55 patients the age at presentation was 1 month (n=19,34%), 2 months (n=17,31%), 6 months (n=7,13%) and more than 6 months (n=12,22%). According to our results, the risk of abortion could be higher 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 among women with recurrent pregnancy losses. In addition, 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 THROMBOSIS
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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 assess any trends or patterns of HAT incidence and characteristics over time. Methods: This study evaluated 2202 VTE episodes across our hospital group, identifying 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 and any additional thrombotic risk factors identified. 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 represented 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 obstetrics 18.4%, general, vascular and GI surgery 12.2%, urology 4% and gynecology and gynaecology 4%). Not all surgeries had an index admission. 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 pre-existing medical conditions and there were no thrombotic events. Of 394 HAT cases with sufficient data, 80 (20.3%) had a preventable cause, 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 undergoing standard patient >90kg. Of those HAT cases deemed unrepreventable, 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/Conclusion: HAT rates remain stable and the majority are thought clinical and by current treatments. Key errors inoviated cases are failure to perform a timely VTE risk assessment and action with appropriate thromboprophylaxis. Full integration of electronic patient records with electronic prescribing modules may reduce further these errors.

E1599 THE QUALITY COMPOSITION OF SOLUBLE FIBRIN MONOMER COMPLEX FRACTION FOR ACUTE AND POST ACUTE ISCHEMIC STROKE PATIENTS
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Background: Inconclusive studies are needed to clarify the real contribution of F12 C46T to thrombosis and pregnancy losses on prospectively selected patients.

Methods: We retrospectively analyzed all the homozygous F12 C46T cases diagnosed in our laboratory from January 2015 to January 2017. Allelic discrimination PCR with TaqManmgB probes was performed to detect homozygous individuals for 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), arterial thrombosis, familiar history of 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% asatric, 4.1% other. Decreased factor XII plasma levels were found in 61 (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 60% of women with recurrent losses. One (43%) or more than one (46.7%) 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: Among the 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 higher 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 among women with recurrent pregnancy losses. In addition, further studies are needed to clarify the real contribution of F12 C46T to thrombosis and pregnancy losses on prospectively selected patients.
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 HiLoad 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 of 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.

E1600 EVALUATION OF A RAPID NANOPARTICLE-BASED LATERAL FLOW IMMUNOASSAY (STIC EXPERT HIT) FOR THE DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA IN A CARDIOHOSPITAL G. Soufla1,*, M. Katafygioti1, S. Georgantis1, T. Kanellopoulou1, T. Kostelidou1

1Department of Haematology, Blood Transfusion Unit and Coagulation and Haemostasis, Onassis Cardiac Surgery Center, Athens, Greece

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 (e.g. during a PTCI). Laboratory testing for the presence of IgA, IgM and IgG or IgG only antibodies against PF4/Heparin (namely HIT antibodies) along with the 4Ts scoring system (Thrombocytopenia: Time of platelet count fall, Thrombosis, Other cause of thrombocytopenia) is used to evaluate the probability of HIT syndrome. At the Onassis Cardiac Surgery Center the method for routine laboratory testing for HIT comprise Enzyme-linked Immunoassay testing for IgG, IgA, IgM H/PF4 antibodies and Heparin-Induced Platelet Aggregation assay for the presence of platelet activating antibodies.

Aims: We evaluated a rapid nanoparticle-based lateral flow immunoassay (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.

Methods: Stic Expert HIT, a rapid-nanoparticle based lateral flow immunoassay was performed on plasma from 35 patients from July 2016 until January 2017 and the reading was done independently by two different technicians or bio-}

The diagnosis of HIT was confirmed when both H/PF4 ELISA and the 4Ts scores were positive.

Results: In 22 cases the Stic Expert was negative for the presence of IgG H/PF4 antibodies in the patients’ plasma and HIT syndrome was easily excluded in combination with the ‘4Ts’. In the rest 13 cases the rapid test provided doubtfull results that were considered as positive and then H/PF4 ELISA was performed. Following the ELISA test, 10 out of the remaining 13 patients were found negative for the presence of IgG H/PF4 antibodies, whereas 3 patients were found positive with a relative low O.D. value (0.400). The last 3 patients that were negative for the presence of IgG H/PF4 antibodies by ELISA were found not to have HIT syndrome in combination with the ‘4Ts’ scoring system.

Summary/Conclusions: In conclusion the Stic Expert HIT was useful for the quick exclusion of HIT (along with the 4Ts scoring system) when emergency HIT diagnosis is needed in 34% of the cases and then H/PF4 ELISA was performed in the rest 13/25 (66%) of the cases, laboratory testing for HIT was much more complicated and time consuming since ELISA or other assays (i.e.HIPA test) had to be performed. Nevertheless all 13 patients were found not to suffer from the HIT syndrome with the ‘4Ts’ scoring system.

E1601 AUDIT OF ‘DOOR TO NEEDLE’ TIME IN ADMINISTRATION OF PROTHROMBIN COMPLEX CONCENTRATE TO PATIENTS REQUIRING URGENT REVERSAL OF NOVAFUSION TREATMENT G. Elshafie1,*, N. Smith1

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Background: Anticoagulants are used to treat or prevent thrombotic events but their more worrying side effect is major haemorrhage. The British Committee for Standards in Haematology (BCSH) recommend reversal of major life-threatening bleeding by giving in both VKAs and DOACs with Prothrombin Complex Concentrate (PCC).

Aims: We aim to reduce delays in the administration of PCC in our trust and introduce the term ‘Door To Needle’ time (DTN) in the context of anticoagulant reversal.

Methods: We analysed the DTN in bleeding anticoagulated patients defined as time from recognition of haemorrhage to PCC administration. In Heart of England NHS Foundation Trust between May and July 2016, 29 patients were included; 19 patients were taking Warfarin and 10 taking DOACs. All patients received PCC (Beriplex®).

Results: Sixty-nine percent of patients were male and 31% female. The majority (69%) of patients were treated for stroke prevention in AF and 24% had a history of VTE. The two commonest major haemorrhage types were cerebrovascular (including intracranial and subdural haemorrhage) in 36% and gastrointestinal bleeding in 39%. The remaining indications (25%) were pre-urgent procedure/surgery, and soft tissue haematoma. The average time for recognition of haemorrhage was 3 hours 20 minutes (range 4 minutes to 21 hours 27 minutes), and the DTN was 4 hours 50 minutes (range 33 minutes to 13 hours 24 minutes), which means an estimated average of 6 hours 27 minutes (range 2 hours 49 minutes to 13 hours 59 minutes) between hospital admission and receiving PCC. Six of the total number of patients died within 30 days of hospital admission, 4 taking on Warfarin and 2 taking on DOACs.

Summary/Conclusions: This audit demonstrates the continuing delays between recognition of major life-threatening bleeding events and receiving PCC since previous audits despite raising staff awareness. We plan to introduce the term DTN in the context of anticoagulant reversal, store PCC in the emergency department pharmacy cupboards (as a POM) as opposed to blood bank, and introduce a reporting system ‘Serious Hazards of Warfarin (SHOW)’ which may further reduce delays, morbidity and mortality.

E1602 THE IMPORTANCE OF PLATELET MEMBRANE FLUIDITY AND OXIDATIVE STRESS IN THROMBOTIC COMPLICATIONS ACQUIRED BY CHRONIC MYELOPROLIFERATIVE NEOPLASMS PATIENTS V.M. Popov1,*, M. Andreescu2, T. Savopol2, E. Kovacs2, H. Bumbea3, A.M. Vladareanu3

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Background: Patients with chronic myeloproliferative neoplasms (MPNs) and chronic myeloid leukemia (CML) have a variety of structural and functional abnormalities of platelets. Many of them have thrombotic or hemorrhage complications. Platelet function is influenced by changes in membrane fluidity (MF) which has an important role in the expression of platelet receptors, modulating the activity of protein membrane.

Aims: The importance of reactive oxidative species (ROS) in alteration function of platelet membrane and expression of platelet receptors in patients with MPNs and CML.

Methods: We present a retrospective study on 36 cases MPN (20 Jak2-positive MPN) and 24 CML admitted in Colentina Clinical Hospital Bucharest. The determination of platelet membrane fluidity was performed by fluorescence anisotropy measurements using as marker 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate (TMA-DPH). We analyzed the fluorescence anisotropy of platelet membrane and correlate the result of with a

gists.
different kind of treatment. Production of ROS was examined using fluorescein diacetate (FDA) and Fluorolog spectrofluorometer. Platelet receiver expression was analyzed by flowcytometry method using 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 than the JAK-negative group. Median value for JAK2 positive group 147.2 ± 10% 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 than the patient who have taken association Hydroxyurea and Anagrelide; 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 value 136 (124.4-147.8) P<0.04. No differences of fluorescence anisotropy was observed between the group of patients who received JAK inhibitor (Jakavi) or Hydroxyurea. The CD42b expression is low in patients versus controls (median: 17.87% vs 94.16%, P<0.03). 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= 94.13%; CD 41=71.13%) versus controls (median: CD 61=98%; CD 41=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 mutation 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 higher patients lot and need to be checked any correlation between formation of fluidity membrane production ROS and expression of microparticles platelet derived.

E1603
USE OF ROTATIONAL THROMBOELASTOGRAPHY TO PREDICT CENTRAL VENOUS CATHETER RELATED VENOUS THROMBOSIS IN CHILDREN: PRELIMINARY RESULTS
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Background: Central venous catheters (CVCs) have been widely used in hospitals for the intravenous administration of chemotherapeutics, contrast media, and other drugs in pediatric age group. However, CVCs are also a cause of venous thrombosis. 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 evaluate coagulopathy. Aims: We aimed to predict CVC related venous thrombosis via ROTEM parameters in pediatric age 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 platelet (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 most 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.

Summary/Conclusions: In this study we reported our preliminary results. We detected thrombosis only in one patient and according 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.

E1604
THE POTENTIAL ROLE OF ANTI NEOPLASTIC DRUGS IN THE PREDICTION OF THROMBOTIC RISK IN ONCOLOGIC PATIENTS IN ADDITION TO THE KHORANA SCORE
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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. Is primary TP in newly diagnosed cancer patients starting chemotherapy (CT), a risk assessment tool (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 lecitidoid, platin and genticabine 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 anti-neoplastic treatment of oncoologic patients diagnosed of pulmonary embolism (PE) from December 2010 until December 2016 at the Complejo 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 oncolgic 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 anti-neoplastic 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 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 median time from the treatment beginning and EP diagnosis was 3 months (0-46).

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Stage</th>
<th>Khorana score</th>
<th>Risk group</th>
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Summary/Conclusions: Nearly 2/3 of Khorana intermediate risk patients developed a PE while on anti-neoplastic treatment and inside this group over 50% were treated with well-recognized high thrombotic-risk drugs. The inclusion of anti-neoplastic drugs in a predictive thromboembolic model in oncoologic 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 oncoologic patients receiving outpatient chemotherapy treatment.

650 | haematologica | 2017; 102(s2)
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 THROMBOTIC SYNDROMES

Background: TTP, HUS and other thrombotic microangiopathy are rare, complex clinical syndromes which are characterized by thrombocytopenia, microangiopathic haemolytic anemia, (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 measure 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 Thromb Haemost 2017).

Results: In our series, the causes and number (%) of MAHA were TTP-HUS (18, 42.9%), autoimmune disorder-associated MAHA (13, 31.1% 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 Vincristine. 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=5) 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. The overall 1 year survival of the entire cohort is 74% which is comparable to the Oklahoma registry. The mean length of hospital stay was 30 days (median 27, SD±20.8), with 1 death and 2 patients in EX included who were ventilated, the mean stay was 42.7±25 days (median 35, SD±20.8) for patients with MAHA, 61.0±29.7 (median 50, SD±25.9) for PEX and 27.9±18.6 (median 21, SD±12.9) for patients with HUS. In treatment group, absolute number and percentage of lymphocyte in WBC decreased significantly after rHuEPO administration from 1798.7±439.0 /µl, in absolute number (p=0.019), and from 33.2±8.57% to 25.7±11.2% (p=0.03) respectively. Regarding B cell subsets, absolute number of naïve B cell and IgD-CD27- B cell in total B cell did not change. These suggested that just one administration of rHuEPO influenced human immune system, especially via reduction of B cell proliferation.

Conclusion: We analyzed peripheral white blood cell subsets in patients who received one bolus administration of recombinant human erythropoietin (rHuEPO) 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 rHuEPO (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 rHuEPO administration. The absolute number and percentage of lymphocytes in WBC decreased significantly after rHuEPO administration from 1798.5±425.0 /µl to 1798.7±439.0 /µl, in absolute number (p=0.019), and from 33.2±8.57% to 25.7±11.2% (p=0.03) respectively. 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, in absolute number (p=0.019), and from 33.2±8.57% to 25.7±11.2% (p=0.03) respectively. Regarding B cell subsets, absolute number of naïve B cell and IgD-CD27- B cell significantly decreased from 171.3±39.5 /µl to 153.0±48.2 /µl (p<0.01), and from 16.7±13.0 /µl to 12.9±7.7 /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 naïve 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.
Methods: A retrospective review of medical records was performed of 44 patients with hematologic malignancies and aplastic anemia who request bloodless treatments in Soonchunhyang university hospital.

Results: Of 44 patients, 48% were men (n=21) and 52% were women (n=23). Mean age was 70.4 years, with a male-female ratio of 1:2, and the range was 16-87. Thirty one patients (70%) were acute leukemia, 15 (34.1%) patients with chronic myeloid leukemia (CML), 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 of real impact of preoperative hematological assessment and optimization of anemic patients in terms of decreasing blood cells transfusions.

Methods: 226 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 etiology. The primary outcome was the response to therapy defined as reaching the Hb level >12 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 and low 26% (gynecology, orthopaedic surgery 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 3% 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 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.

Table 1. Units transfused in hematology clinic

<table>
<thead>
<tr>
<th>RBCs</th>
<th>AML</th>
<th>Lympho</th>
<th>MPN</th>
<th>Myeloma</th>
<th>MDS/AA</th>
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</thead>
<tbody>
<tr>
<td>800/0</td>
<td>700/0</td>
<td>600/0</td>
<td>500/0</td>
<td>400/0</td>
<td>300/0</td>
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</table>

E1610

RED BLOOD CELLS (RBC) AND PLATELET (PLT) TRANSFUSIONS IN TRANSPLANTED AND NOT-TRANSPLANTED PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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2Hematology Department, Papageorgiou General Hospital, Thessaloniki, Greece

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 Unit (bloodless transplant unit- BMTU).

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 major 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 transplantations (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 time of the hospital stay was 18 (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 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 hematology clinic, the underlying disease was correlated with transfusion needs in RBC 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
Acute lymphoblastic leukemia - Biology

PB1611

BOTANICAL ALKYL HYDROQUINONE HQ17(3) EXERTS CYTOXICITY TO T(9;22) PHILADELPHIA CHROMOSOME SUP-B15 ALL CELLS THROUGH INDUCING ENDOPLASMIC RETICULUM STRESS, AUTOPTOXY, AND ALF INDUCTION

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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 chemotherapy and tyrosine kinase inhibitor treatment. Development of new adjuvant therapeutics will provide great value. HQ17(3) [10(Z),13(E),15(E)-heptadecacontatetraynyl hydroquinone] isolated from sap of the lacquer tree showed potent cytotoxic effect within 24 hours at micromolar concentration on several ALL cell lines, including TKI (imatinib, IM)-resistant Ph+ B-ALL cell line SUP-B15 cells, but spare normal PB leukocytes, and were non-toxic in experimental rats after 28-day HQ17(3) injection. Thus HQ17(3) presents as a potential anti-leukemic agent and serves a model for design anti-leukemic regimen. We previously showed HQ17(3)-induced rapid cell demise, characterized by oxidative stress, loss of membrane integrity, mitochondrial membrane potential disturbance and nuclear DNA fragmentation. Neither pan-caspase inhibitor nor Nec-1 (RIP-1 inhibitor) protected against HQ17(3) treatment. SUP-B15 cells from HQ17(3)-induced cell death. The cell death program elicited by HQ17(3) is caspase-independent, and is different from the RIP1-mediated controlled necroptosis.

Aims: To investigate the characteristics of, and the molecular pathways involved in the HQ17(3)-induced non-classical death on VHR-ALL and cell death pathways for the VHR-ALLs.

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 (3-MA) were used in combination with HQ17(3) in some experiments. Acidine orange stain and confocal microscopy are used to visualize the changes of acidic vesicles. Autophagic flow in response to HQ17(3) was revealed by aggregating of ectopically expressed EGF-FP-LC3. Western blot analysis were used to p- eIF2a, ER chaperone Grp78, spliced RIP1 to investigate the relationship with RIP1 in some experiments.

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 lysosomal membrane permeabilization (LMP) as caspase inhibitors did not attenuate the cell death. HQ17(3) enhanced autophagy as revealed by aggregation of ectopically expressed EGF-FP-LC3. Inhibition of autophagy by Bafilomycin A1 or knockdown 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 AIFF in a similar fashion with mitochondria disturbance and caspase-independent cell death thereafter. Nuclear accumulation of apoptosis inducing factor (AIFF) was revealed by fluorescence microscopy.

Summary/Conclusions: In Ph+ ALL SUP-B15 cells, HQ17(3) acts in multi-facet: a) lead to oxidative stress and perturb mitochondria membrane permeabilization, b) induce ER stress and calcium mobilization to mitochondria, c) induce apoptosis as revealed by shRNA to control the VHR-Ph+-ALL cells refractory to conventional high dose chemotherapy and TKI regime.

PB1612

TARGETED MUTATIONAL PROFILING OF CHILDHOOD AND ADULT ACUTE LYMPHOBластIC LEUKAEMIA PATIENTS

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Background: Acute lymphoblastic leukemia (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. We analyzed DNA samples from 34 de novo ALL patients (17 cALL and 17 aALL) using TruSeq Amplicon – Cancer Panel (TSCAP) 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 2500 × per amplification region. Ten genes were discarded due to insufficient coverage, therefore we analyzed 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 mutational frequencies to public databases and global cancer mutation rate results.

Methods: We set out to comprehensively characterise the second ALL, including conventional G-banding and fluorescence in situ hybridisation (FISH). The

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: 10–13). Overlapping was of 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: 1–9). 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 HNF1A gene coding for transcription factor involved in both blood development and kidney development. AQ1 signal 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. By combining both DNA sequence data and RNA expression data, it is possible to find new key signaling pathways, primarily Ras/RKT and Notch pathways. This study contributes to knowledge of ALL mutational landscape, leading to better understanding of molecular basis of ALL and better stratification and treatment of ALL patients.
Results: The profiling analysis showed G-banding 36,XX,-2,-3,-del(5)(q31q35),-7,-12,-13,-14,-15,-16,-17,13q(11-13)/46,XX(7). Extensive FISH analysis confirmed the diagnosis of low hypodiploidy ALL. This result was in line with the reported association between TP53 gene mutations in 90% 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 genetcs, FISH probes were used on archived diagnostic slides. Careful selection of probes demonstrated that the original leukemia sample contained two co-existing clones – one low hypodiploid clone (with an identical pattern of loss) and a gain of chromosomes as the second ALL) and one clone resembling a doubled up/tripled 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 (R3), to which she has shown a promising response. She is considered for allogeneic bone marrow transplantation using an unrelated donor.

In hindsight, the treatment regimen used for the initial ALL was incorrect. If it had been established that she had low hypodiploid ALL the first time around, she would have been allocated the most intensive regimen within the trial. Nevertheless, she maintained remission status for 5 years with low intensity treatment and ironically 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 WITH MULTIPLEX IMMUNOHISTOCHEMISTRY

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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 intensive 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 dyes for six markers and nuclei simultaneously enabling cytometric analysis at cell-level resolution. Marker panels included T and B lymphoid cells, NK and dendritic cells, macrophages as well as myeloid derived suppressor cells. Furthermore, we analyzed immune checkpoint molecules (PD1, LAG3, OX40, TIM3, CTLA4) and their ligands (PD-L1, PD-L2, HLA-G, HLA-ABC) alongside with various activation markers (granzyme B, CD45RO, CD25, CD57, CD27). After the data was collected, the cells were segmented and quantified with the image analysis software CellProfiler and the cell analysis software FlowJo.

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 leukemia 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]) of CD4+ T-cells, 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 PD-1-positive CD8+ T cells in Ph+ ALL BM vs 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 (27.1% [IQR 21.6-33.25] vs 18.5% [IQR 14.8-21.9]), of CD4+ cells, p=0.0001), but no difference was observed in the proportion of OX40-positive CD8+ T-cells (p=0.49).

Summary/Conclusions: Multiplex IHC enables ample cytometric evaluation of different immune cell subtypes in their original microenvironmental context of the bone marrow. The TMA format not only allows analysis of tens of BM samples in parallel but also serves as a retrospective, easy-access archive for any follow-up studies. Ph+ ALL BM is characterized by a decrease in the CD4+/CD8+ ratio and an increase in the proportion of CD4+CD45RO+ T-cells in comparison with the non-leukemic controls. The proportion of PD-1-expressing T cells is also elevated. However, the heterogeneity between patients is marked. The analysis of other marker panels is presently ongoing, as well as correlation to clinical and treatment outcome parameters.

PB1615

CDKN2A/p16INK4A DELETION IS NOT A POOR PROGNOSIS PREDICTOR IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED ACcORDING TO PROTOCOL RALL-2009

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Background: CDKN2A/p16INK4a deletion is a frequent cytogenetic abnormality in acute lymphoblastic leukemia (ALL), ranging from 18% to 45%. In pediatric group of patients p16INK4a deletion was associated with T-cell ALL phenotype and poor event-free survival. The prognostic impact of CDKN2A/p16INK4a deletion in adult ALL patients appear controversial.

Aims: To evaluate the prognostic impact of the CDKN2A/p16INK4a deletion in adult patients with acute lymphoblastic leukemia.

Methods: We present the results of the CDKN2A/p16INK4a deletion in 110 adult patients with newly diagnosed Philadelphia–negative ALL, which were treated by RALL-2009 (NCT01193933) in our center since June 2009 till September 2016. Patients characteristics: the median of age was 26 years old (range 15-54), the median white blood cell (WBC) count was 16.9×10⁹/L (range: 0.4-785×10⁹/L), the median blasts cells count in the bone marrow (BM) was 84.4% (range: 0-98). Sixty-five (59%) of the 110 patients had a B-cell phenotype, 42 (38%) had a T-cell phenotype, 3 (2.7%) patients - biphenotypical ALL. Interphase fluorescence in situ hybridization (FISH) was performed for detection CDKN2A deletion, TEL/AML1, MLL rearrangement, MYC (8q24.21) translocation, TP53 deletion, IAMP21.

Results: 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 PD-1-positive CD8+ T cells in Ph+ ALL BM vs 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 (27.1% [IQR 21.6-33.25] vs 18.5% [IQR 14.8-21.9]), of CD4+ cells, p=0.0001), but no difference was observed in the proportion of OX40-positive CD8+ T-cells (p=0.49).

Summary/Conclusions: Multiplex IHC enables ample cytometric evaluation of different immune cell subtypes in their original microenvironmental context of the bone marrow. The TMA format not only allows analysis of tens of BM samples in parallel but also serves as a retrospective, easy-access archive for any follow-up studies. Ph+ ALL BM is characterized by a decrease in the CD4+/CD8+ ratio and an increase in the proportion of CD4+CD45RO+ T-cells in comparison with the non-leukemic controls. The proportion of PD-1-expressing T cells is also elevated. However, the heterogeneity between patients is marked. The analysis of other marker panels is presently ongoing, as well as correlation to clinical and treatment outcome parameters.
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 biphenotypic 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=0.000), with high 9p21 (37%) of genes (LDH level the median is 3062 EU/L, p=0.0004) and no associating with CR and relapse incidence was found. We didn’t revealed relationship between CDKN2A deletion and MILL, TEL/AML1 rearrangement, MYC translocation, TP53 mutation and IAMP21. CDKN2A deletion didn’t have statistically significant impact on outcome of patients. The five-year OS and EFS was 65% and 40% with and without deletion was 85% and 76% (p=0.05), 90% and 85% (p=0.05), respectively. OS for T-ALL patients with and without deletion was 90% and 80% (p=0.03), 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 associ-ation between deletion of the CDKN2A gene and with known cytogenetic pro-gnostic factors. However patients with T-cell ALL and CDKN2A deletion had a more aggressive initial clinical features (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). Addi-tionally, we set up a genomic qPCR to screen for CDKN2A and CDKN2B dele-tions in a larger cohort of patients (n=53).

Results: qPCR results showed that most of the 9p21 losses corresponded to homozygous deletions in both genes (36%, 19/53), while heterozygous deletions corresponded to 5.7% (3/53) and different CNA status between CDKN2A and CDKN2B was to 28% (15/53) of the samples. Global alterations in CDKN2A/B locus were found in a high proportion of patients. Differently obtained CNA results confirmed the findings obtained by qPCR. The resolution of the array allowed us to distinguish between homozygosis in CDKN2A and heterozygosis on CDKN2B. The FISH analysis corroborated the homozygous deletion in the CDKN2A/B locus in all the cases analyzed. With that, we ask for clinical implications. CDKN2A/B locus abnormalities, mainly homozygous deletions, were found 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 patients. Supported by 2014 SGR225 (GRE) from CERCA Programme/Generalitat de Catalunya, and by funds from Josep Carreras International Foundation, “la Caixa” Foundation and Celgene Spain.

PB1817 BUTEIN KILLS ACUTE LYMPHOBLASTIC LEUKEMIC CELLS IN VITRO AND IN VIVO THROUGH FOXO3A AND CASPASE-DEPENDENT APOTOTIC 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 mecha-nisms of butein on ALL were investigated.

Methods: We examined the rate of apoptosis of CEM-C7 (T-ALL), CEM-C1 (T-ALL), MOLT-4 (T-ALL), RS4-11 (B-ALL) cell lines and primary ALL blasts from 20 children using Annexin V and monochromatic fluorescence microscopy.

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 established the xenograft mouse model to examine the anti-leukemic effect of butein in vivo.

Results: Butein was found to significantly decrease 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 path-ways.

PB1618 GENOMIC LANDSCAPE AT DIAGNOSIS AND RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Childhood acute lymphoblastic leukaemia (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 develop 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 ana-lyzed by multichannel flow cytometry, standard karyotype and FISH. SNP array (Affymetrix®) performed on cryopreserved cells at diagnosis and relapse investigated copy number alterations (CNA) and loss of heterozygosity (LOH). TP53 mutation was studied on paired samples by Sanger sequencing.

Results: The median age in the EGIL classification between diagnosis and relapse. Diagnostic cytogenetics prognosis was good for 3 children, poor for 3 (AmpAML1, KMT2A and complex cytorex) intermediate for 2 (normal karyotype). Three patients showed additional karyotypic anomalies at relapse. SNP array showed a mean of 10 CNA and 0.6 LOH modulations at relapse. Seven of the 8 patients pre-sented modulation in CNA and LOH during evolution with a median of 4. Some anomalies observed by cytogenetics were refined by SNP analysis, notably all chromosomal gains and losses were recovered and precisely located. More-
over, a (4;8) translocation was found to be more complex with 7 and 8 RNA on chromosomes 4 and 8. Patients with the most RNA and LOH also had a complex karyotype. Anomalies were observed in hot spot regions in 9p (comprising 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, EBF1, 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 genes of interest. The 2 patients with cytogenetic intermediate prognosis would thus probably have been considered for a more intense than prophylactic 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, deoxynucleobase triphosphates are unprotected, 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 that are responsible for removing damaged nucleotides is Nudix hydrolase15 (NUDT15). NUDT15 inhibits incorrect DNA synthesis and induction of apoptosis. NUDT15 inhibits incorrect cell proliferation and apoptosis through catalysis of thiopurine hydrolysis. Tanaka et al. claimed that, besides TPK variants in Japanese patients, there might be other possible additional factors that may influence thiopurine toxicity. They reported that NUDT15 variants are more specific to Asian population when compared to European people. As far as we know, this is the first study on screening of possible variants in the first exon of NUDT15 in Turkish children with precursor B-cell acute lymphoblastic leukemia (Pre-B ALL).

Aims: In this study, our aim was screening of gene variants in first exon of NUDT15 in pediatric group of patients diagnosed with Pre-B ALL.

Methods: Our study group was composed of 83 patients aged between 1-15 years. The 83 patients were diagnosed with Pre-B ALL diagnosed at Lönsä Hospital. DNA samples were isolated by MagNa Pure system. First exon of NUDT15 was amplified by PCR reaction. After PCR purification, sequencing was performed.

Results: After screening of first exon of NUDT15, we detected two variations. First variation was intronic insertion which was defined as rs38311098 (c.158+52_158+53insGGGGCGTGCGCAGAGGGACGATCTC). The other intronic variation was defined as rs79687000 (c.158+117C>T). rs3831098 was detected in 17/64 children (19 girls and 45 boys, median age 8.25 years). In total, 48 patients are living in the first/second complete remission. Relapse of ALL was associated with Mantel Cox test was done.

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 p-B ALL.

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 a 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 TCR 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 (24XCyte/2XCyte Probe Kit; MetaSystems) or CGH-SNP array (SurePrint G3 Cancer CGH+SNP 4x180K, Agilent). For OS and EFS Kaplan-Meier analysis was 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 TCR 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 p-B ALL.

Supported by grants RVO-VFN64165, GACR-P302/12/G157 and NPU I nr. LO1604

PB1621
ADULT PRIMARY ACUTE LEUKEMIA SAMPLES WITH CHROMOSOMAL TRANSLocations GROW WELL IN IMMUNODEFICIENT MICE, BUT ARE DIFFICULT TO TRANSDUCE WITH LENTIVIRUSES
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Background: Acute leukemia (AL) is a severe disease of the hematopoietic system and associated with a poor outcome for patients. Patient derived xenograft (PDX) mouse models provide an attractive tool to engrat and grow primary tumor cells. In contrast to culture growth, samples can be monitored in a consistently
microenvironment. This powerful tool provides the baseline for further experiments like preclinical treatment trials or biology studies. While good engraftment rates were published for primary pediatric ALL samples, engraftment rates of adult ALL samples might be inferior, but remain largely elusive.

**Aims:** This study aimed to determine engraftment and growing ability of primary adult AL samples in immunodeficient mice. Genetic engineering was performed to evaluate transduction efficiencies by lentiviruses in PDX AL cells.

**Methods:** Primary adult ALL and AML samples were transplanted into NSG mice in the absence of total body irradiation. Both frozen and fresh patient material was used. Human CD45 and human CD38 were stained in blood to monitor successful engraftment. Mice were sacrificed before coming down with leukemia. lsolated cells from bone marrow and spleen were analyzed by flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of fluorochrome markers and flow cytometry. Results: Engraftment and growth was successful in NSG mice in 12 out of 15 primary adult ALL samples. Frozen samples showed a longer median engraftment time compared to fresh samples. In particular, fresh samples could already be detected with an average time of 75.29 days. Generally, the engraftment 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-F4 ALL samples. Adult ALL PDX samples with chromosomal translocations showed very low transduction 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 transduction, transduction efficiency for AML rearranged samples was high with up to 60%. These values are similar to non-rearranged ALL samples having transduction rates between 30% up to 80%.

**Summary/Conclusions:** In summary, we observed a high engraftment rate of primary adult ALL samples in immunodeficient mice which was above what we anticipated from the literature. Adult PDX samples can be transduced with lentiviruses with identical high transduction efficiency as pediatric samples, with an age independent exception of AL PDX cells with BCR-ABL or MLL translocations.

**PB1622**

SYNERGIC CHEMOTHERAPEUTIC EFFECT OF MENADIONE COMBINED WITH EPIGALLOCATECHINE-3-GALLATE OR DOXORUBICIN IN A HUMAN CELLULAR MODEL FOR ACUTE LYMPHOCYTIC LEUKEMIA A. Garaiman1,2,*, I. T. Tofelean1, R. M. Babes1, C. Ganea1, I. Baran1

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**Background:** Epigallocatechine-3-gallate (EGCC) and menadione (vitamin K3; MD) are known as potent apoptogens in cellular models for acute lymphocytic leukemia (ALL) – Jurkat T cells.

**Aims:** The goal of this study was to explore the chemotherapeutic potential of MD combined with EGCC or DOX, and to determine whether there is a synergic interaction between these agents that could significantly enhance their antitumoral effect in a cellular model of ALL. We investigated the antiproliferative effect of EGCC and DOX combined alone or in combination EGCC:MD and MD:DOX respectively on human leukemia Jurkat lymphoblasts. Some underlying cellular mechanisms were also scrutinized.

**Methods:** Cells suspensions of Jurkat lymphoblasts were treated at various concentrations of EGCC, MD and DOX. Clonogenic survival was evaluated as the colony forming capacity in 96-well plates. Cell cycle and apoptosis/necrosis were determined by flow cytometry using the fluorescent indicators propidium iodide and Annexin V-FITC/7-AAD, respectively. Determination of oxidative stress and mitochondrial polarization was performed by spectrofluorometry, using the fluorescent probes CM-H2DCFDA and JC-1, respectively.

**Results:** Logistic clonogenic survival established a depolarizing effect at 17 µM of MD (Hill coefficient h = 3.17) and mitochondrial calcium in a dose-dependent manner (IC50 = 97 µM, h = 2.53). Furthermore, data show that there is no correlation between the level of mitochondrial calcium ([Ca2+]m) and mitochondrial membrane potential (ΔΨm) (Pearson correlation coefficient r = -0.100) or between [Ca2+]m and reactive oxygen species (r = 0.437) generated by the EGCC-induced depolarization. Fluorescence induced by treatment with EGCC alone, MD alone and EGCC:MD in combination was 172, 101% and 387%, respectively, suggesting that EGCC and MD interact with the second specific target (the mitochondria). The rate of DOX induced apoptosis correlated well with DOX generated oxidative stress. MD augmented this effect, enhancing the proapoptotic effect of DOX most likely by increasing the affinity of DOX for nuclear DNA.

**Summary/Conclusions:** Our results support the notion that the combinations EGCC:MD and MD:DOX exert a strong synergic antiproliferative effect in human leukemia Jurkat cells and encourage further studies to test the clinical utility of this association in ALL therapy.

**PB1623**

FOCAL ERG DELETIONS AND DUX4 FUSIONS IN CELL LINES DERIVED FROM B CELL ACUTE LYMPHOLASTIC LEUKEMIA H. Quentmeier1,*, R.A. MacLeod1, C. Pommerenke1, H.G. Drexl1

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**Background:** DUX4 has recently been presented as new oncogenic driver in B cell acute lymphoblastic leukemia (pre-B-ALL) of adolescents and young adults [1]. Translocations of DUX4, especially those with the IGH locus led to expression of the corresponding fusion gene. DUX4 then triggered the expression or a novel isoform of the ETS transcription factor ERG in pre-B-ALL [2]. Focal deletions of exons 3-9 were a second cause for short ERG variants. Up to 7% of pre-B-ALL showed deregulated expression of both genes, DUX4 and ERG [2].

**Aims:** We set out to find pre-B-ALL cell lines with DUX4 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 DUX4 or ERG. Using RT-PCR (TaqMan probe HS03079790_g1) we were able to consistently detect ERG exon 6. Determination of alternative ERG exons was performed by RT-PCR.

**Results:** Genomic PCR showed that 2/6 pre-B ALL cell lines (NALM-6, SUP-B15) tested carried deletions targeting ERG exon 5. Results of DUX4 qRTPCR (TaqMan probe Hs00236770_m1) were positive for NALM-6 and SUP-B15. However, cell line SUP-B15 did not express DUX4 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 DUX4. Cell line NALM-6 is presented as model system for DUX4/ERG pre-B-ALL.

**References**


2 Zhang J, McCastill K, Vidyarthi R, DUX4 translocations. Genomic PCR was performed to detect focal ERG deletions. qRT-PCR showed expression of alternative ERG exon 6, transcriptional target of DUX4.

**PB1624**

NATURAL HISTORY OF SECONDARY MULTILINEAGE PROLIFERATION WITH MONOSOMY 7 FOLLOWING TREATMENT OF RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA J. Buls1,*, A. Pobudejcka-Pieniazek1, L. Siedek2, A. Lopez2, M. Jara-Acevedo3, A. Kowalska-Pawlak1, A. Sosnala1, A. Ortafo1, T. Szczepanski1

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**Aims:** We present a case of a 11-year-old boy with the history of relapsed ALL treated with an anthracycline and irinotecan chemotherapy. Despite the absence of a minimal residual disease, he developed late combined ALL relapse (BM and testis). Unilateral orchidectomy was performed for aberrant expression of ERG and fusion of DUX4 and ERG, indicating the presence of secondary lineage involvement.

**Methods:** Most of the pediatric samples expressed DUX4-induced gene (DUX4-IHG) translocation and ERG mRNA as potential indicators for the development of secondary lineage proliferation. NALM-6 was the only cell line expressing the DUX4 protein. Like- wise, the alternative ERG transcript with alternative exon 6 was observed in NALM-6 only.

**Summary/Conclusions:** In conclusion, focal ERG deletions in pre-B-ALL cell lines (2/6) occur at similar frequencies as in the primary tumor. Cell line NALM-6 carries the DUX4-IHG translocation, expresses the DUX4 protein and an ERG mRNA variant including the alternative exon 6. ERG deletions were present in two cell lines NALM-6, SUP-B15. However, cell line SUP-B15 did not express DUX4 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 DUX4. Cell line NALM-6 is presented as model system for DUX4/ERG pre-B-ALL.

**References**

2. Zhao J, McCastill K, Vidyarthi R, DUX4 translocations. Genomic PCR was performed to detect focal ERG deletions. qRT-PCR showed expression of alternative ERG exon 6, transcriptional target of DUX4.

Background: T cell acute lymphoblastic leukemia (T-ALL) is a hematopoietic clonal malignancy caused by the malignant transformation of T lymphocyte driven by gene mutation. The prognosis of T-ALL is poor and early relapse is common.

Aims: We aimed at looking for specific and effective therapeutic target for T-ALL and eventually cure this form of leukemia by targeted therapy.

Methods: Bone marrow mononuclear cells (BMMC) are collected from bone marrow samples of T-ALL patients, including at initial presentation (n=46), during maintenance treatment, the patient showed persistent pancytopenia.

Results: By real-time PCR. Changes in the expression level of c-FLIP L mRNA is significantly higher in patients at initial presentation and relapse, compared to those at complete remission and healthy control. The expression level of c-FLIP L mRNA is associated with patient risk stratification, white blood cell count, serum LDH level, serum HBDH level, CD45, SIL-TAL1 fusion gene, complex karyotype and disease outcome. c-FLIP L mRNA was used as a prognostic marker in T-ALL.

Summary/Conclusions: The c-FLIP L mRNA expression level is abnormally high in T-ALL patients both at initial presentation and at relapse. The expression level of c-FLIP L mRNA is associated with risk stratification, white blood cell count, serum LDH level, serum HBDH level, CD45, SIL-TAL1 fusion gene, complex karyotype and disease outcome. c-FLIP L mRNA was used as a prognostic marker in T-ALL.

PB1626

CYP1A1 AND CXCL12 GENE POLYMORPHISMS IN CHILDHOOD ACUTE LYPHBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most common type of childhood leukemia and represents one third of all pediatric malignancies. Due to the high survival rate (35-80%), there is a number of children relapse and for them the outcome remains poor. Epidemiological studies that examined possible risk factors of acute leukaemias, proved that genetic factors play a crucial role in leukemogenesis. Recent genetic association studies on cancer risk, have focused on the effects of single nucleotide polymorphisms in genes that regulate inflammation and tumor suppression such as chemokines and P450 cytochrome. Chemokines induce the motility of endothelial and tumor cells. CXCL12, a chemokine expressed in various tumors, binds to chemokine receptor 4 (CXCR4) and is considered to play an activating role in the conversion of environmental chemicals into carcinogens. The above gene contains two important single nucleotide polymorphism, CYP1A1*2A (rs1801157) and CYP1A1*2C (rs1048943) with Poly-Merase Chain Reaction (PCR); The PCR products were digested with the restriction enzymes MspI and BsrDI for CYP1A1. Descriptive statistics and logistic regression analysis were used to examine for differences between children with ALL and controls.

Results: In the CXCL12 loci, the frequencies of AA, AG, and GG genotype were 3.45%, 93.1% and 3.45% in children with ALL, 13.3%, 60.0%, 26.7% in children control group and 4.17%, 45.83% and 50.0% in adult control group respectively. In the CYP1A1 loci, the frequencies of AA, AG, and GG genotype were 13.3%, 86.7% and 0% in children with ALL, 90.0%, 10.0%, 0% in children control group and 81.6% and 16.4% and 2.0% in adult control group respectively. No statistical significant differences in CXCL12 polymorphism were revealed between children with ALL and healthy groups using logistic regression analysis. Regarding CYP1A1 loci, we detected a positive association for the AG polymorphism and ALL [OR: 37.7 (95% CI: 10.81, 131.37), p<0.001 and OR: 58.5 (95% CI: 9.66, 354.12), p<0.001 using only the children's control group].

Summary/Conclusions: A higher frequency of CYP1A1 heterozygote allele was observed among children with ALL compared to controls, whereas no differences were observed regarding CXCL12 polymorphisms. Future studies in larger populations are needed in order to specify the role of the above polymorphism in childhood ALL.

PB1627

INTRACHROMOSOMAL AMPLIFICATION OF CHROMOSOME 21 IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: A RARE SUBTYPE

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Background: Intrachromosomal amplification of chromosome 21 (iAMP21) is a rare genetic abnormality of chromosome 21. It is considered to function as a monoclonal malignancy. Several iAMP21 cases have been documented in adults but to date, there is only one reported pediatric case [1]. We report a case of a 13-year-old boy with iAMP21 acute lymphoblastic leukemia (ALL) diagnosed at the pediatric clinic of Pécs University Hospital.

Methods: We performed cytogenetic analysis and assessed the expression of the clade-specific marker MLLT10 (11q23) and CD45 (11q23), and compared the results to an iAMP21-negative case.

Results: We identified a t(1;21)(q22;q22) chromosomal translocation with MLLT10/CD45 fusion gene and a complex karyotype with 14 additional abnormal alterations. The patient was treated with a modified BFM chemotherapy protocol and achieved complete remission. The follow-up at 14 months showed no evidence of leukemia.

Summary/Conclusions: This is the second pediatric case of iAMP21 ALL reported in the literature. Our case highlights the importance of genetic aberrations in pediatric ALL and the potential role of iAMP21 as a new therapeutic target.
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 RB1 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 common ALL 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 in 3 of 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 Lymphoblastic Leukemia (r/r ALL) (Topp et al.). Extra-medullary 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-medullary 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 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 therapeutic chemotherapy (COG/ALL 2007 regimen) (Domenich 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 maculopapular 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 blastic cells were observed on the second’s. One month later, another skin biopsy showed a CR without lymphocytic infiltrate. The medullar CR was confirmed at the molecular level (MRD negative). The first patient received allogeneic 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 infiltration’ by Blinatumomab in r/r B-ALL with cutaneous infiltration suggesting promising activity in extra-medullary relapse. Further studies are required to confirm a Blinatumomab-based strategy in extra medullary 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 IMMUNOGLOBULIN 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; or 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 (1–105). 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.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Early MRDneg</th>
<th>Late MRDneg</th>
<th>Late MRDpos</th>
</tr>
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<tbody>
<tr>
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<td>M/F</td>
<td>M/F</td>
</tr>
<tr>
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<td>16–73</td>
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<td>&gt;10 × 10⁴/μL</td>
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<td>MRDneg</td>
<td>MRDneg</td>
<td>MRDpos</td>
</tr>
<tr>
<td>CR</td>
<td>100%</td>
<td>72.9%</td>
<td>20%</td>
</tr>
<tr>
<td>DFS (m)</td>
<td>&gt;105</td>
<td>&gt;105</td>
<td>&gt;105</td>
</tr>
</tbody>
</table>

* WBC risk: B >3 × 10⁴/μL, T >10 × 10⁴/μL.
** Cytogenetic risk: Hypodiploidy, complex karyotype, MLL rearrangement.
*** Achievement of remission after 2 cycles of chemotherapy.

MRD, minimal residual disease; PCR, polymerase chain reaction; IgH, immunoglobulin heavy chain; M, male; F, female; WBC, white blood cell; CR, complete remission.

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 PEGASPARAGINE FOR THE TREATMENT OF NEWLY DIAGNOSED ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Asparaginase is a component of a multi-agent chemotherapy regimen widely used in the treatment of acute lymphoblastic leukemia (ALL). Since 2006, pegasparaginase (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 limits of the National Library of Medicine data base 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 68.5% (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% (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 asparaginases.

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 P. Minetto1,*, N. Bisso1, F. Guolo1, M. Clavio1, E. Coviello1, D. Guardo1, N. Di Felice1, F. Canale1, L. Mancon1, F. Balleri1, M. Miglioli1, R. M. Lemolli1, M. Gobb1

1Clinic of Hematology, Department of Internal Medicine (DIMI), University of Genoa, IRCSS AOI San Martino-IST, Genoa, Italy

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 synergetic 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 ephegmocly, a macroscopic picture resembling sinusoidal occlusive disease, ultrasonography showed acute liver steatosis. All 3 patients received concomitant therapy withidarubicin, 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 ofidarubicin (p = 0.046, HR 1.45) were administered. Steroids therapy determined a borderline increase in toxicity risk (p = 0.068, HR 1.50). No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, mitoxantrone 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 carbapenems and azoles (Table 2). No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, mitoxantrone and 6-mercaptopurine (Table 1).

Table 1. Costs in € per patient (with and without HSCT) by quarter after relapse

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Index quarter (relapse)</th>
<th>II</th>
<th>III</th>
<th>IV</th>
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<tbody>
<tr>
<td>N</td>
<td>mean</td>
<td>% increase</td>
<td>cost</td>
<td>N</td>
</tr>
<tr>
<td>ALL without HSCT</td>
<td>18</td>
<td>14.74</td>
<td>16%</td>
<td>10</td>
</tr>
<tr>
<td>ALL with HSCT</td>
<td>31</td>
<td>28.01</td>
<td>78%</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>35.21</td>
<td>82%</td>
<td>20</td>
</tr>
</tbody>
</table>

Results: Of the total 116 incident adult ALL patients identified, 29 (25%) were determined to have had a relapse and 11 understand HSCT after relapse (38%). Patients with an HSCT appear to incur higher cost than those without HSCT in each of the quarters after relapse was diagnosed (Table 1), with the highest in the first quarter after relapse, but decreasing in subsequent quarters. Inpatient cost accounted for the majority of the cost for the first three quarters for both HSCT and non-HSCT patients, but for HSCT patients. The number of patients in the HSCT cohort remained relatively stable, while the non-HSCT cohort had only half the patients left by the third quarter post relapse.

Summary/Conclusions: Our data show that the toxicity profile of PEG-ASP in adult patients is overall manageable. However, serious warnings emerge from our experience. Concomitant drugs and their timing of administration may play a crucial role in significantly contributing to PEG-ASP hepatic toxicity. In order to attempt to reduce toxicity, anthracyclines with shorter half-life, i.e. daunorubicin instead of idarubicin, should be used. A particular attention should be paid when administration of concomitant antibiotic therapy is required.

PB1633

COST OF CARE FOR ADULT PATIENTS WITH RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA WITH AND WITHOUT HEMATOPOIETIC STEM CELL TRANSPLANT IN GERMANY

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Background: Adult ALL is a rare but frequently fatal disease. Many patients who respond to initial therapy experience a relapse. For relapsed ALL (rALL), hematopoietic stem cell transplant (HSCT) is a potentially curative treatment option. HSCT is associated with added costs, however, which could impact overall healthcare budget.

Aims: This retrospective observational study aims to determine the cost of care and the impact of HSCT on total cost for adult rALL patients from a German payers’ perspective.

Methods: A German claims database with a representative sample of approximately 7 million individuals insured within the German statutory health insurance and continuously observable over a period of 6 years was used as data source. For these data, 70 adult patients (18 years and older) with a new diagnosis of ALL (ICD-10-GM code: C91.0*) between January 1, 2011 and December 31, 2015 and a relapse after remission to initial treatment were identified. Mean health care cost per patient per quarter, the smallest unit of time available in the database, was determined by whether or not patients had an HSCT after relapse. Costs were considered from the perspective of the German statutory health insurance and included costs for prescription medicine as well as outpatient and inpatient healthcare encounters.

Results: Of the total 116 incident adult ALL patients identified, 29 (25%) were determined to have had a relapse and 11 underwent HSCT after relapse (38%). Patients with an HSCT appear to incur higher cost than those without HSCT in each of the quarters after relapse was diagnosed (Table 1), with the highest in the first quarter after relapse, but decreasing in subsequent quarters. Inpatient cost accounted for the majority of the cost for the first three quarters for both HSCT and non-HSCT patients, but for HSCT patients. The number of patients in the HSCT cohort remained relatively stable, while the non-HSCT cohort had only half the patients left by the third quarter post relapse.

Table 1. Costs in € per patient (with and without HSCT) by quarter after relapse

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<td>Total</td>
<td>49</td>
<td>35.21</td>
<td>82%</td>
<td>20</td>
</tr>
</tbody>
</table>
Summary/Conclusions: The results of this study inform the magnitude of cost in Germany associated with adult rALL patients who do not have 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 biologic 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 cases. Cytogenetic information was unavailable in 45% of cases due to low follow-up time in 36 patients was 22 months (range: 0,5-84 month). 4

Summary/Conclusions: The frequency and prognostic impact of mutation status of IKZF1 deletions in patients with Ph-neg ALL patients with IKZF1 mutations and without

PB1636

THE FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF IKZF1 DELETIONS IN ADULT PH-POSITIVE AND PH-NEGATIVE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED IN RUSSIAN ACUTE LYMPHOBLASTIC LEUKEMIA STUDIES

Background: The frequency 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-negative ALL, but not in patients with Ph-positive ALL, suggesting that therapeutic interventions 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 56 adult patients (median age 27, range 17-66; 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 leukemia (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 cent multiplex polymerase chain reaction according to the procedure described by [Aurelie Caye et al, Haematologica, 2013]. DNA for PCR was extracted from leukemia 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% (p=0.77), relapse - free survival (RFS) - 25% and 33.3% (p=0.88), respectively.

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-
pos ALL and, on the contrary, more favorable for BCR-ABL1 - neg ALL, though not statistically significant. Having or not IKZF1 mutations, all BCR-ABL1-pos ALL patients are candidates for allogeneic hematopoietic stem cell transplantation (allo-HSCT). Regarding BCR-ABL1-neg ALL: though the group of patients is small, we can suggest that IKZF1 mutation did not appear to influence survival due to different chemotherapy principal in RALL — 2009 — non-intensive but not-interruptive therapy with low numbers of HSCT.

Future prospective studies should focus on this issues.

Figure 1. Relapse-free survival.

PB1637
GMALL BASED PROTOCOL, USING NATIVE E. COLI L-ASPARAGINASE, IMPROVES SURVIVAL OF ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN BRAZIL
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1Instituto Nacional de Câncer, Rio de Janeiro, Brazil

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. BCR/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-based 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-cell 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 HR 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 MDR 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 which may be related to several aspects: socioeconomic impairment, inadequate supportive care for more intensive therapies and ineffective cancer care network. Future prospective studies should focus on this issues.

Figure 1.

PB1638
THE INVESTIGATION OF RELATIONSHIP BETWEEN COL1A1 AND FOK1 GENE POLYMORPHISMS AND DEVELOPMENT OF TREATMENT-RELATED SKELETAL COMPLICATIONS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
M. Erdem1, Ö. Tüfekçi1, S. Kızıldağ2, S. Yılmaz Bengoa1, D. Kızmaçoğlu1, B. Eroğlu Filibeli3, H. Ören1
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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, corticosteroid 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, PCR, Fok1 gene and coloidal 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% for 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...
OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBlastic LEUKEmIA TREATED WITH PEDIATRIC PROTOCOL: MONOCENTRIC STUDY

I. Frikha1,*, M. Medhaffer1, S. Hedioui1, R. Kharrat1, M. Chaari2, G. Kassa2, M. Ghrib2, H. Bellaila1, I. Ben Amor1, F. Kallek1, C. Kallek1, M. Elloumi1

1Hematology, 2Laboratory of Hematology, Hedi Chaker Hospital, Sfax, Tunisia

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, Cyto-genetic results), we studied the protocol results: relapse, progression, risk of good and aggressive leukemias, and its stressful treatment, not only high: VHR, remission rate, death rate, relapse rate and 5 years survival (overall and event free EFS).

Results: Seventy two AYA ALL were treated with the pediatric protocol. The patients were 45 males and 27 females (SR=1:6). A WBC>100 G/l was noted in 32%, T ALL blasts were 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 (33%) of the patients from protocol were eligible for allogeneic stem cell transplantation (SCT), among them 15 patients had a familial donor and 10 patients were allotransplant (42%) and only 4 patients still in CR (2 patients died by GVH and 4 patients relapsed). Relapse was observed in 22 patients (32%), among them 12 during the first year of treatment. The median follow up was 101 months (8.4 years).

Summary/Conclusions: The results of this pediatric based study show that response to therapy and prognostic in adolescent and young adults were better than those treated with adult protocols and tolerability of chemotherapy is acceptable. However OS and EFS, better than adult ALL treated by adult protocol (OS=14%, EFS=14%: local study) was not satisfactory because of the high toxic mortality rate.

SEVERE PSYCHIATRIC DISTURBANCES DURING THERAPY IN PEDIATRIC ALL

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Background: Psychiatric disturbances are not uncommon in patients with cancer. Their pathogenetic mechanisms are variable and comprise consequences of the therapy, underlying disease, as well as personality characteristics. These disturbances are frequently associated with the use of corticosteroids, which is an essential component of the treatment for children and adolescents with Acute Lymphoblastic Leukemia (ALL).

Aims: This 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 aged 9-16 years with newly diagnosed acute lymphoblastic leukemia. 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 11 protocols. One protocol (AR1) was treated 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 prednisolone.

Summary/Conclusions: Severe psychiatric disturbances are not infrequent in children and adolescents receiving treatment for ALL. Awareness 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. 
Department of Clinical Institute Fundeni during 2010-2017 and received chemotherapy according to protocol ALL BFM 1985 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 treated according protocol ALL BFM 1995 and 7 patients were treated according to protocol ALL BFM 2002. M/F ratio was 4/5. Patients had at 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 (CT and MR imaging) 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 LEUKEMIA 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 the different risk factors, genetics, and improvement in diagnosis and treatment. Armenia is considered to be a more ethnically nation.

Aims: We aimed to quantify the incidence of and mortality from acute leukemias among children population in Armenia and their variation with gender, age, year of diagnosis.

Methods: In this work we included children diagnosed with de novo acute leukemia, 0–18 years of age from 2006 to 2016. The initial data for this survey have been derived from ambulance/dispensary cards, hospitalization journals, and clinical data from the Registry of Blood Diseases at the R. Yeolyan Hematology Center, Yerevan, Armenia. The data has been supplemented by the data from the Registry of Oncological Diseases of the V. Fanarjyan NCO, as well as from death certificates. The demographic data has been obtained from the National Statistics Board of Republic of Armenia. The obtained data has been statistically analyzed using EPI INFO-2002 program.

Results: A total of 277 cases of childhood acute leukemia were identified, 174 (62.2%) males and 103 (37.8%) females. The overall incidence of leukemia was 3.4 per 100,000 children-years. The higher incidence rates were noted in 2006-2007, 2011 and 2014–2015. The lower incidence rates were noted in 2007, 2012 and 2010 (accordingly 4.0, 4.0 and 3.9), and the lower rates in 2011, 2014, 2009 (accordingly 2.4, 2.4 and 2.9). Three are registred regions in Armenia-Lori, Vayots Dzor and Tavush with higher incidence rate (respectively 0.020, 0.020 and 0.018 per 100,000 children-years. The lowest incidence rates were noted in Armavir and Ararat (0.018 in 2006-2016). The 5-year survival rate was 72%, 100%, and 100% among children diagnosed at 3–7, 7–13, and 13–18 years of age, respectively. The results indicated that the children diagnosed between ages of 3 and above had the lowest risk of mortality and higher survival rates.

Summary/Conclusions: In this first general population study to describe the incidence of and mortality from childhood acute leukaemias in Armenia during 2006-2016. It forms the basis for quality assessment of acute leukaemia treatment in Armenia and offers a unique opportunity for population-based research. Age at diagnosis remained to be a crucial determinant of the survival and treatment in Armenia and offers a unique opportunity for population-based research. Age at diagnosis remained to be a crucial determinant of the survival and treatment in Armenia and offers a unique opportunity for population-based research.
Results: Eight of 21 (38%) patients exhibited an isolated t(4;11) translocation. Additional chromosome abnormalities (ACA) 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 aberrations, 58% vs 13%, 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-ABL1 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
DERMATOLOGIC 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 (PDGFRα, PDGFRβ, C-KIT, CSF1-R, FLT3). But other TKI such as BCR/ABL 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 tyro- sine 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 pt (pt 4), without molecular remission on dasatinib and nilotinib ther- apy, 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 trans- plantation.

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 two weeks of sorafenib treatment in pt 1. 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 treat- ment gray hair observed. Skin rash with pigmentation grade I evolved in pt 3 after 12 weeks of therapy. Pt 4 had grey hair after 12 weeks second-generation TKI (bosutinib) treatment. Palmos-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 ponatinib treatment in pt 5 (with portisiosis anamnesis). Pt 6 developed localized maculo-papular skin rash grade I after 5 weeks of sorafenib treatment. Despite of all patients developed der- matological side effects, temporarily discontinuation of TKI therapy was required in only three (50%) cases. In the other cases the treatment was con- tinued. 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 doses reduction or interruption of TKI therapy led to complete resolution 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.

PB1647
CYTOKINE RELEASE SYNDROME AFTER THE FIRST INTRATHECAL CHEMOTHERAPY IN A PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH AN EARLY MENINGEAL RELAPSE
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Background: Central nervous system (CNS) is a frequent site of recurrence in childhood acute lymphoblastic leukemia (ALL), and Triple Intrathecal Therapy (TIT) with Methotrexate (MTX), Cytarabine (ARA-C) and hydrocortisone, at the moment of the mainstay of the treatment of CNS relapse. Severe neurotoxicity is well known TIT complication, usually related to repeated infusions and neurotoxic concomitant systemic drugs.

Aims: We describe a case of a massive acute leukoencephalopathy after only one TIT in a 5-year-old child with an early isolated CNS relapse of ALL (26 months after the first diagnosis), rapidly proceeding to comatose status.

Methods: At admission for disease restaging at the end of first-line trial, the child showed physical and neurological examination completely negative, such as haematological, biuroral and ultrasound findings. The cerebrospinal fluid (CSF) appeared turbid and liquoral pressure increased. CSF count showed 8100 cells/µl: morphology and flow cytometry confirmed an early isolated CNS relapse. Due to the abnormal pleocytosis, TIT administration associated with oral dexamethasone was suddenly performed without any other concomitant chemotherapy. To prevent acute toxicities from tumor lysis syndrome, the patient received hydration, allopurinol, acetazolamide and prophylaxis of seizures with levetiracetam. After few hours from TIT, the child developed severe headache followed by skin urticarial rash, high blood pressure and hallucinations, rapidly evolving in flaccid paralysis of lower extremities. A brain resonance (MRI) showed diffuse areas of hyperintensity of white matter, par- ticularly in lombar region. The MRI pattern was interpreted as diffuse grade IV leukoencephalopathy of probable toxic nature. The child, 30 h after TIT, was transferred to intensive care unit for progressive ascending paralysis and respiratory distress that required intubation. During the following days, other three diagnostic lumbar puncture were performed that showed significant reduction of blasts cells (20, 10 and 0 cells /µl, respectively).

Results: Patient persisted in deep coma for 5 days, until he restart a sponta- neous breathing. After waking up, the child showed rapid neurological amelio- ration; the seizures reappeared, especially during nights, spontaneous noises, hand and feet fingers. The subsequent MRI highlighted improvement of hyper- intensity at midbrain, brainstem and bridge brain areas and spinal cord with persistence of altered signals in subcortical white matter. The visual evoked potentials were normal and the motor and sensory conduction velocity appeared slowed without axonal damage; EEG showed slow waves spread. At the moment, after three week from severe neurological complication, the child is fully awake, moving all four limbs, but requires motor and phoniatric rehabilitation. Systemic chemotherapy with high-dose MTX and IT ARA-C is restarted without any additional neurotoxicity. Dosage of CSF levels of interleu- kin 6 and its soluble receptor is ongoing.

Summary/Conclusions: Although leukoencephalopathy following IT MTX or ARA-C administration are described, the severity and rapidity of event’s onset, associated with CSF remission after a single TIT administration, suggests us that neurotoxicity could be related to massive blast cytolysis with subsequent patient’s death. This syndrome is a frequent complication of blinatumomab or chimeric antigen receptor T-cells administrations. The CSF IL-6 dosing could clarify the patho- genesis of the event.
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 hematopoietic disorders 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/day for 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 2x109/L (blasts 15%), 8x109/L (blasts 30%) and 18x109/L (blasts 61%), while platelet count was reduced in 2 cases (61x109/L and 65x109/L). Coagulation tests were normal (fibrinogen median level 380 mg/dl). Severe hypofibrinogenemia developed between 6 and 15 days after beginning treatment and lasted between 4 and 48 days. Fibrinogen nadir ranged from 47 to 100 mg/dL (median 61 mg/dL); reduced plasma fibrinogen levels at functional tests were also confirmed to immunological assays. During fibrinogen nadir, D-dimer was positive in all patients, but stable compared to the outset. Antithrombin, coagulation factors, activated partial thromboplastin and prothrombin time, common liver function 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 the treatment to reduce the risk of the 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). As positive are determined mutations with variant allele frequency (VAF) at least 2%.

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 FLT3-ITD 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 CEBPA 35/231 (15.2%). The analysis also identified mutations in rarely mutated genes U2AF1 (9/231 [3.9%]), SF3B1 (9/231 [3.9%]), EZH2 (3/231 [1.7%]), U2AF1 (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 NRRAS.

Figure 1. Distribution of genes with VAF 32% in AML cohort. Each column represents one patient (n=231). Each row represents one gene described on left, on the right is shown the number of patients with mutation in the gene and its percentage from the total cohort. The color of the squares represents the status of the gene: red – single mutated, blue – double mutated, black – triple mutated, white/grey – no mutation.

Supported by Ministry of Health of the Czech Republic, grant nr. 15-25809A, and by project MUNI/A/1106/2016. All rights reserved.

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. 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 expressing 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. IGF2BP1 was confirmed to be a novel downstream target of LIN28B via let-7 miRNA in AML. Notably, silencing LIN28B led to slow tumor growth in vivo.

Summary/Conclusions: In conclusion, these results uncover a novel mechanism of an important regulatory signaling, LIN28B/let-7/IGF2BP1, in leukaemogenesis 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 negative) as a marker of remission and treated with intensive chemotherapy (2009-2015). Bone marrow (188) and peripheral blood (277) samples were analyzed from complete remission (MRD 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. Patients in relapse had MRD NPM1 >0.02%. Cox regression was used for univariate analysis.

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+/CD38/KG-1a and primary AML CD34+ cells as research object.

Results: In this study, we demonstrated that AT101, a BH3 mimetic pan-Bcl-2 inhibitor, was significantly and effectively cytotoxic towards CD34+/CD38−/CD123+/CD38/KG-1a 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 partipate in DNA damage generation and initiation of DNA damage response, ultimately leading to apoptosis. However, they fail to completely 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−/CD123+/CD38/KG-1a 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−/CD123+/CD38/KG-1a 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 γH2A.X, with DNA damage accumulation and repair defects. Co-treatment with chidamide and DNA-damaging agents IDA gave rise to production of γH2A.X, with DNA damage accumulation and repair defects. Further analysis on AML patients’ clinical characteristics revealed that the ex vivo efficacy of chidamide in combination with IDA in primary CD34+ samples was significantly correlated to peripheral blood WBC counts at diagnosis, while status, LDH level, karyotype had no effect, indicating that the combination regimen of chidamide and IDA could rapidly diminish tumor burden in a patient with R/R 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
Abstract withdrawn.

PB1657
NEW CANDIDATE GENES USEFUL TO PREDICT THE RISK OF RELAPSE IN ACUTE PROMYELOCYTIC LEUKEMIA
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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: A relapse is a very 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. We analyzed blasts in samples obtained from Bone Marrow with Single Nucleotide Polymorphisms Array Cytoscan HD.

Results: We compared copy number alterations in both relapsed patients with alterations detected in the pool of 20 newly diagnosed APL and we found specific signatures of CNVs for each patient. There were several copy number alterations related to each patient: the first patient presented gain of ROBO2, GRIP1, CTNNB1, SOX6, PBX1, GRIK2, CDKAL1 and loss FAP1, CREBBP, SBF1; the second patient gained copy of ROBO1, MAPK10, CADP52, APBA1 and loss of GRIP1 and MYB. Subsequently we focused our attention on ROBO and GRIP1 genes because they were altered in both relapsed patients: ROBO proteins are associated to K channels while GRIP1 is involved in various critical functions, for example in androgen receptor binding, beta-catenin binding, glucocorticoid receptor binding, and it is also a regulator of glutamate metabolism, a well-known pathway in Leukemic Stem Cells.

Summary/Conclusions: By the analysis of ROBO 1-2 and GRIP1 at the diagnostic stage we could establish a different and strict follow-up program for patients with these alterations.

Acknowledgement: ELN, AIL, AIRC, prog. Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project, HARMONY.

PB1658
THE EXPRESSION OF SALL4 AND BMI-1 GENES IN MYELOID LEUKEMIA
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Background: Sal-like protein 4 (SALL4) and B-cel specific moloney murine leukemia virus integration site-1 (BMI-1) genes are stem cell genes that modulate stem cell pluripotency and may play a role in leukemogenesis. Leukemic stem cells (LSCs) have been implicated in being the origin of the leukemic blast, therapy resistance and relapse.

Aims: The current study aimed at characterizing the expression pattern of SALL4 and BMI-1 genes in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), in patients who have achieved complete remission (CR), and in CML disease progression.

Methods: Real-time polymerase chain reaction was used to assess the gene expression patterns in 106 myeloid leukemia patients; 54 de novo AML (43 at time of diagnosis, 11 in CR), and 52 CML (31 in chronic phase (CP), 11 in deep molecular response (MR) & 10 in accelerated/blastic phase (AP/BP), and in 21 non malignant bone marrow samples.

Results: SALL4 gene expression was increased in AML patients, AML-CR, & CML-CP (median= 5.180, 4.604 & 14.125 respectively). No significant differences were observed between de novo AML and AML in CR patients. CML-CP patients showed a significantly higher percentage of patients with a high SALL4 expression as compared to both CML- MR and CML-AP/BP (p<0.033). BMI-1 gene expression was not found to be increased in any of the patient groups. Summary/Conclusions: Our data describe altered SALL4 gene expression in different phases of myeloid leukemia. The role of BMI-1 gene needs further delineation to determine its significance.
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 haematological malignancy characterised by the over proliferation and block in differentiation of clonally altered leukemia potential bone marrow stem cells such as S100A8 could assesses the progression and remission of AML.

Aims: S100A8 and S100A9 (Cal 2+ 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 Chevassut 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 mRNA 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 expression was found to be variable amongst the samples but also in comparison to OCI-AML3 and THP-1. 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 monocyte 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 driving the leukemia. Further work may provide more detailed insight into the mechanisms of action and potential use of S100A8 and S100A9 in AML prognostic markers.

PB1659
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) is constrained by the inability to sequence certain genes due to high GC content, large indels and low complexity regions. Here we present the coverage and variants generated from numerous hybridisation-preparation in combination with a SureSeq myPanel™ NGS Custom AML panel. The library was then sequenced using a 2x150 bp read length protocol on Life Technologies.

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 SureSeq 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-duplicated depth in excess of 2000x as well as ITD’s 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).

PB1612
PROTEOMIC APPROACH TO IDENTIFY MOLECULAR TARGETS OF HALOFUGINONE IN ACUTE MYELOID LEUKEMIA
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Background: Halofuginone (HF) is a halogenated derivative of Febrifugine, which is a molecule isolated from the plant Dichroa febrifuga. It has been demonstrated that Halofuginone exhibits anti-fibrotic, anti-cancerogenic, anti-inflammatory and pro-apoptotic effects. Previously, we have reported that treatment with HF has anti-leukemia properties in vitro and in vivo in acute promyelocytic leukemia (APL), reducing tumor growth through the induction of apoptosis and by stimulating the synthesis of the TGF-β protein and activating its downstream targets. In addition, HF presented anti-angiogenic effects by modulating the level of pro and anti-angiogenic factors including VEGF. However, it is unknown whether HF could cooperate with other courses of acute myeloid leukemia (AML) and HF targets were not determined yet.

Aims: Evaluate the anti-angiogenic effect of HF on other AML subtypes by APL and investigate its targets using a proteomic approach.
Methods: AML cell lines Kasumi-1, THP-1, MVA11, 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 were determined for each cell line. We used the Proteome Profiler TM Array – Human-Phospho-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, THP-1 cells were transplanted into SCID mice and treated with vehicle or HF (mean OS of 70.5 and 68 days, respectively; p = 0.24). In contrast, the mean OS for NSG mice transplanted with Kasumi-1 cells with 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 synthate (eNOS) and Signal transducer and activator of transcription 3 (STAT3 Y705), 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: Aberrant 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 signature of each mutation.

Methods: We accomplished DNA hydroxymethylation and methylation profiling in 12 AML samples at diagnosis and in CD34+ cells of 3 healthy controls by MethylationEPIC array (Illumina) covering aprox. 850 000 CpGs. AML samples were selected 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 (CEQX) to convert DNA through oxidative bisulfite (oxBS) and bisulfite (BS) treatment. This approach allows us to determine whether CpG is methylated or rather hydroxymethylated.

Results: We performed hierarchical clustering analysis of oxBS β-values (corresponding to DNA methylation levels) of 830 304 CpGs (with detection P<0.05) and observed clear separation of 4 groups according to mutational status – DNA methylated (CD34+), IDH1+ activated, IDH2+ and CD34+ samples relative to CD34+ normals. Interestingly, the positive DNMT3A+ or IDH1+ and CD34+ normal strongly suggests that there is a cumulative effect of these two opposing mutations (Figure 1). We found out that genes hypermethylated in IDH1+ samples are enriched for genes from HOX gene family (P<0.05). Genes that are hydroxymethylated are often genes that are methylated in IDH1+ patients. In addition, we detected a subgroup of CpGs assigned to HOX2A, HOX4A, HOXA10, HOXB3, HOXC4 and HOXD3 genes that are hypermethylated in IDH1+, hypomethylated in DNMT3A+ and normally methylated in DNMT3A+ and IDH1+ samples relative to CD34+ normals. Clustering of DNA hydroxymethylation values (resulting from subtraction of oxBS β-values from BS β-values) resulted into the same 4 main clusters as shown for DNA methylation data. DNMT3A+ patients displayed the lowest hydroxymethylation levels from all patients. 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.

PB1664
RNA-MEDIATED CORRECTION OF ABBERRANT DNA METHYLATION AT THE P15 LOCUS
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Background: P15 (a.k.a cell cycle dependent kinase inhibitor 2B; CDKN2B; INK4B) is a methylation sensitive gene located on chromosome 9p21 and commonly found silenced during Myelodysplastic Syndrome (MDS) progression to Acute Myeloid Leukemia (AML). P15 encodes for a cyclin-dependent kinase inhibitor increasingly expressed during granulomonocytic maturation (Teofili et al., Exp Hematol 2000). P15 deletion or promoter methylation has been shown to independently correlate with disease progression and poor patient prognosis (Tien et al., Br J Hematol 2001). Additionally, P15 expression was also sensitive to regulation by myeloid-specific transcription factor PU.1 (Schmidt-Blood 2004). As MDS evolution to AML includes both myeloid proliferation and blocked differentiation stages, restoration of the natural P15 transcript will provide not only valuable information regarding disease progression but may also alleviate some of their characteristic symptoms.

Aims: Currently available demethylation agents approved for therapeutic applications, e.g. 5-azacytidine and decitabine, have major side effects of high toxicity and non-specific DNA methylation that limit their clinical application. Therefore, the aim of this study is to achieve RNA-mediated correction of the aberrantly methylated P15 locus using small activating RNAs (saRNAs; Li et al. 2006).

Methods: Myeloid Leukemia cell lines HL-60, KG1a, and K562 were screened for basal p15 expression by western blotting and qRT-PCR. As the p15 locus is also often deleted, deletion of the locus was assayed for by PCR and by Fluorescent In Situ Hybridization. The methylation status of P15 was shown to be inversely correlated with ANRIL (Antisense Non-coding RNA in the INK4a Locus) expression (Kotake et al Oncogene 2010), p15 and ANRIL gene expression were measured in parallel. HEK293 cells serve as positive control in all studies. SaRNAs were designed against the proximal promoter, first exon, and intron regions of the P15 gene body. SaRNAs were introduced to cell lines through electroporation, and re-activation of the locus was measured at the transcript level by qRT-PCR and protein level by western blotting. Changes in P15 promoter level methylation were determined by Methylation Specific PCR.

Results: Transfection of saRNAs into the HL60 cell line showed upregulated p15 expression 24 and 48 hrs post-transfection. Analysis of ANRIL after saRNA-transfection showed no concomitant changes, suggesting locus-specific activity of the saRNAs. Future experiments will elucidate the mechanisms of saRNA activation of P15 gene expression and genome-scale specificity of saRNA treatment.

Summary/Conclusions: There is much interest in using RNA molecules as a therapeutic tool (Kole et al., Nat Rev Drug Discovery 2012; Reebey et al., Hepatology 2014). Introduction of such an approach offers greater advantages over
Results: In apoptosis analysis, a synergic effect was detected for all 4 cell lines, similarly cell cycle evaluation showed a significant accumulation of cells in 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 5±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 76±2 for their combination (P<0.005). The THP1 cells did not show any significant modulation in the proliferation. We decided to focus our study on (f: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 direct 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 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 known 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 TP33G 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. The median levels of functional nuclear p53 protein are reduced in NPM1 and FLT3 ITD mutant samples. TP35 encodes a tumor suppressor protein which consists of transactivation, DNA-biding and oligomerization domains. Due to alternative splicing it may exist in 2 different protein isoforms, p53β and p53γ, without oligomerization domain. The alternative splicing is associated with cell cycle regulation, 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 TP33beta and TP33gamma expression levels.

Methods: 75 NK AML patients were included in the study. NPM1, CEBPA and FLT3 gene mutations were analyzed by direct sequencing. TP33β and TP33γ 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, TP33β and TP33γ 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 TP35β was much higher (ΔΔCt 43,11) than TP35γ (ΔΔCt 10,85; p<0,05). Furthermore, expression level of TP33γ 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 TP33β or TP33γ expression. We also classified patients according to median expression value of TP35, 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 TP33 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 TP33 isoform expression and mutations in CEBPA gene. It may be hypothesized that a changed sequence of the latter gene might influence TP33 isoform expression and in consequence regulate the cell cycle.
was performed on erythrocyte-lysed bone marrow (BM) samples obtained at the Hospital Clínico, Valencia, and the Hematology, ICO Hospitalet, L’Hospitalet de Llobregat, Hospital Universitario de Gran Canaria Dr. Negrín, Hematology, Hospital de la Santa Creu i Sant Pau, and Hematology, Hospital Universitario de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain.

Background: Whole-genome sequencing has revealed acute myeloid leukemia (AML) as a very complex and dynamic disease. Epigenetic modulation is among the functional categories of the mutational landscape in AML. According to recent reports, suppression of the epigenetic reader BRD4 with small-molecule inhibitors (BET-i) results in antileukemic activity. Clinical trials are being developed, however, so far, identification of those patients that may benefit from this therapy is not possible as changes in mRNA BRD4 levels seem to differ between patients. It has been recently suggested that antileukemic effect of BET-i could be due to c-myc suppression and that also high Bcl-2 levels may target those patients that would benefit of BET-i. We believe that establishing the expression profile of epigenetic modifiers in AML may help in the identification of patients that could benefit from BET-i.

Aims: We wanted to get a better insight regarding the expression profile of epigenetic modulator in AML of intermediate risk by studying: 1) expression levels of EZH2, ASXL1, BRD4, c-myc and Bcl-2 in a consecutive series of AML patients; 2) correlation between mRNA and protein levels; 3) Determining BRD4 binding to the c-myc promoter through chromatin immunoprecipitation (CHIP).

Methods: Our series consisted of 104 consecutive patients with a mean age of 55.8 years (range 15-79 years) diagnosed and treated between 2005-2016 at the Hospital Universitario de Gran Canaria Dr. Negrín with a median follow up of 12 months. Gene expression analysis was carried out through real time PCR in a LightCycler 480 Instrument II (Roche) using GUS a control gene. Results were normalized with a cDNA pool from bone marrow of 10 healthy donors which was introduced as internal control in each experiment. Western blot were performed to determine protein levels for BRD4, c-myc and Bcl2. CHIP studies for BRD4 were carried out in HL60 cell line. For statistical analysis the SPSS (v.15.0) software was used.

Results: ASXL1 levels were positively associated with EZH2 (Pearson’s r= 0.285, p=0.021) and BRD4 with c-myc (Pearson’s coefficient r=0.420, p<0.001). Bcl2 (Pearson’s r= 0.471, p=0.001) EZH2 (Pearson’s r= 0.4655, p=0.008) and ASXL1 (Pearson’s r=0.949, p<0.001). Survival analysis considering 50th percentile as a cut-off value for BRD4 expression indicated that patients with higher levels had a better overall survival (median OS of 27 months, 95% IC 15.1-38.9) compared to those with low expression (median OS 12 months, 95% IC 0.4-23.7), although the association was not statistically significant (p=0.196) probably due to the limited series size. Protein levels of Bcl2 and c-myc correlated with those of mRNA, but not for BRD4, although other antibodies should be tested in order to confirm these results. CHIP analysis in HL60 cell lines confirmed the binding of BRD4 to c-myc promoter.

Summary/Conclusions: The positive association observed between EZH2 and ASXL1 agrees with the fact that both cooperate in the epigenetic repressive complex PRC2. The increased expression of BRD4 with c-myc and Bcl2 is in accordance to the reported binding of BRD4 to the c-myc and Bcl2 promoter enhancer regions and our CHIP analysis also support so. Further studies in a larger series of CEBPAdm cases. Small leukemic populations with B-cells markers are not uncommon in CEBPAdm AML (3/39, 7%).

PB1668

FLOW CYTOMETRY IMMUNOPHENOTYPING IN CEBPA-DM DE NOVO AML. BIOLOGIC AND PROGNOSTIC RELEVANCE.

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Background: CEBPA is a transcriptional co-factor of RUNX1 which play a major role in the fate decisions associated with physiologic myelopoiesis. Biallelic CEBPA mutations (dm) define an homogeneous molecular subgroup which is associated with a favorable outcome. CEBPA mutations may be transmitted in the germ line giving rise to clusters of familial leukemias. Aims: To analyze the immunophenotypic findings assessed by multiparametric flow cytometry in a consecutive series of de novo CEBPAdm AML.

Methods: Thirty nine adult patients with de novo AML and CEBPAdm who were enrolled on the AML-03 and AML-12 protocols of the Spanish CETLAM cooperative group were included in this study. The immunophenotypic analysis was performed on erythrocyte-lysed bone marrow (BM) samples obtained at diagnosis. Antigenic expression of leukemic cells was systematically analyzed by multiparametric flow cytometry using four-color staining. The antigens studied were: CD45, CD34, HLA-DR, CD10, CD20, CD19, CD2, CD33, CD7, CD117, CD66, CD13, CD64, CD36, CD56, CD14, CD123, CD61, CD42b, glycophorin, CD71, CD11b, myeloperoxidase, CD79a, TdT, lymphocyte and lactoferrin. At least 10,000 events/tube were measured. Analytical gates were established according to CD45 reactivity and to FSC/SSC pattern. Positivity threshold was established at 20%. The FACS-DIVA,Paint-a-Gate and Infinicyt software programs were employed for analysis. Amplification of overlapping PCR products covering the whole CEBPA coding sequence followed by Sanger sequencing were used to investigate CEBPA mutations. FLT3-ITD, NPM1, MLL-PTD, WT1 and GATA2 mutations were also investigated by conventional PCR-based molecular methods.

Results: Antigen reactivity was as follows: CD45 (39/39,100%), CD15 (35/39, 90%), CD34 (36/39,92%), HLA-DR (39/39,100%), CD33 (39/39,100%), CD2 (2/39,5%), CD7 (36/39,92%),CD117 (39,39,100%), CD13/37/95,95%, CD56 (39,15%), CD36 (6/39, 15%), CD123/39, 100%), CD14 (1/39,0.02%), CD71 (39/39,97%), myeloperoxidase (38/39, 97%). In nine cases CD36 and/or CD56 expression on leukemic blasts was greater than 20% Those CD36/CD56+ cases had a shorter overall survival and leukemia free survival (see graph). Four out five tested CD36/CD56+ cases also showed GATA 2 mutations. An additional CD36/CD56+ case had a FLT3-ITD. In three out 39 cases (7%) a population showing cytoplasmic CD79a reactivity was detected (8%, 11%,14% of the neoplastic population, respectively). Two of those cases had also a FLT3-ITD.

Figure 1.

Summary/Conclusions: CEBPAdm cases showed an homogeneous immunophenotype with positivity for CD45, CD7, CD34, CD123, CD117, HLA-DR, CD71, CD33, CD13 and CD15. CD36 and/dor CD56 overexpression was detected in a subgrupo 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%).
Results: Image analysis revealed an average of 464 protein spots in PB samples taken at time of diagnosis, and an average of 348 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 correspond 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.

Table 1.

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Summary/Conclusions: The protein expression profile of AML patients changes 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 INTERFERS WITH OVEREXPRESSION OF NUCLEOPHOSMIN 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 toxicity. Nucleophosmin (NPM1 or B23) is a ribosomal protein located in the nucleolus and multifunctional enzyme in cancer-related molecular and protein synthesis. 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 cell cycle distribution in AML cells treated with low or high concentration of cytarabine, an anti-leukemic drug, to predict the mechanisms responsible for AML cells chemoresistance.

Methods: We exposed HL-60 (FAB M2) cells to different concentrations of cytarabine for 48 h, and the cell viability was measured by cell counting kit-8 (CCK-8). The cell cycle distribution was measured by Annexin V-FITC/PI double staining flow cytometry (FCM). The protein expression levels of NPM1, TRF1, TRF2 were measured by western-blotting.

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 was significantly regulated by cytarabine. The protein expression levels of NPM1 and TRF1 were measured by western-blotting.

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.

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 in various diseases, including leukemia. Therefore, targeting telomere-binding proteins is considered as a promising therapeutic strategy for treatment of leukemia.

Aims: We aimed to explore whether quercetin, a natural flavonoids, could regulate telomere-binding proteins expression to inhibit proliferation and induce apoptosis in acute myeloid leukemia(AML) THP-1 cells.

Methods: 1. In vitro: (1) We cultured human AML THP-1 cells. (2) The cells were treated with different concentration of quercetin for 24/48 h, and the cell viability was measured by cell counting kit-8 (CCK-8). (3) The cell cycle distribution was measured by Annexin V-FITC/PI double staining flow cytometry (FCM). (4) The mRNA expression levels of POT1, TRF1, TRF2 were measured by western-blotting. (5) The mRNA expression levels of POT1, TRF1, TRF2 were measured by real-time fluorescent quantitative polymerase chain reaction (RT-qPCR).

2 In vivo: (1) Established AML-NOD/SCID model based on THP-1 cell line in NOD/SCID mice, and treated with optimal quercetin concentration 40mg/(kg*d) for 4 weeks by tail vein injection. (2) We observed the changes of mice survival status, peripheral blood and bone marrow cell morphology and organ histopathology by microscopy before and after treatment with quercetin. (3) Thel cell cycle distribution and apoptotic rate of spleen cells were measured by Annexin V-FITC/PI double staining FCM. The protein expression levels of POT1, TRF1, TRF2 were measured by immunohistochemistry (IHC) staining.

Results: In this study, we found that quercetin significantly suppressed THP-1 cells proliferation in dose- and time-dependent manner. Treatment with quercetin significantly increased the protein expression of POT1, TRF1, TRF2 and G1 phase arrest rate. Furthermore, the protein expression levels of POT1 and TRF1 increased and the protein expression level of TRF2 decreased. The mRNA expression levels of POT1, TRF1, TRF2 were consistent with their protein expression levels, respectively.

Summary/Conclusions: Our results demonstrate that quercetin has anti-leukemia activity. It is mediated by regulating telomere-binding proteins expression of POT1, TRF1 and TRF2. Taken together, our findings support the concept that quercetin is a promising therapeutic strategy for treatment of leukemia.

PB1672

PPARα AGONISTS INHIBIT ADHESION SIGNAL TO ENDOTHELIAL CELLS IN THE DIFFERENTIATION INDUCTION OF RS5-ACTIVE 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 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
signs or symptoms of DS is crucial, however specific biological therapies to counteract the syndrome are still not available. Peroxisome proliferator activated receptor gamma (PPARγ) is a ligand-dependent transcription factor and a member of the nuclear receptor superfamily, which is expressed in normal monocytes, various leukemias, and epithelial malignancies. PPARγ is highly induced in differentiating myeloid cells and subsequently contributes to their differentiation. Differentiation induction of APL cells is associated with increased expression of specific adhesion molecules and inflammatory cytokines, which may promote activation, migration, and adhesion of these cells.

**Aims:** Here, we studied the effect of PPARγ agonists on the adhesion of a human leukemia cell line (HL-60) to endothelial cells.

**Methods:** Differentiation was determined by an increase in reactivity with the CD11b antibody. For the adhesion assay, the Matrigel transwell system was used.

**Results:** HL-60 cells were differentiated into macrophage-like cells by a PKC activator, 12-O-Tetradecanoylphorbol-13-acetate (TPA). During the differentiation of HL-60 cells, PPARγ agonists activate TPA-induced CD11b expression. However, PPARγ agonists completely blocked TPA-induced ICAM-1 expression of endothelial cells, which resulted in the inhibition of adhesion of HL-60 cells to endothelial cells. These responses also were reversed by PPARγ antagonist (GW9662), indicating that PPARγ agonists inhibits the adhesion of the HL-60 cells to endothelial cells through a PPARγ dependent mechanism.

**Summary/Conclusions:** These results suggest that PPARγ agonists inhibit TPA-induced adhesion signal in the between HL-60 cells and endothelial cells, and may control differentiation syndrome in APL patients.
Summary/Conclusions: Expression of p53 assessed by immunohistochemistry is a fast, specific and reproducible 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 CLADRIBINE, HIGH DOSE CYTARABINE AND IDARUBICIN
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Aims:

- To investigate the combination of 2CdA, AraC and idarubicin (CAI)
- To assess the impact of EVI1 transcript levels in Chinese AML patients

Methods:

- Patients with relapsed AML after at least 6 months of remission and 2CdA 0-2 were included. Chemotherapy regime consisted of two courses of 2CdA mg/m²/d × 12, h-1, d-3, AraC 1000 mg/m²/d, h-1, d-3 and idarubicin 8 mg/m²/d, d-1. After 8 patients, the prolonged duration of neutropenia especially in course 2 prompted us to change the protocol by: application of growth factors from day 15 onwards, and 2) omission of idarubicin from the 2nd course.

Results:

- The primary endpoint was the overall remission rate and safety of CAI.
- 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, infection rates are a serious complication warranting intensive supportive care.

PB1677
HIGH EVI HIGH EXPRESSION PREDICTS POOR OUTCOMES IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS WITH INTERMEDIATE CYTOSKELETAL RISK RECEIVING CHEMOTHERAPY ONLY
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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 patients were treated. Decitabine was given in a 5 days-adapted dose of 9 mg/m² for 5 days every 4 weeks.
- Median age was 76 y (69-85), ECOG performance status (PS) was ≥3 in 10.8%. According to “fitness”, 41 pts (89.1%) were defined unfit to chemotherapy aged ≥65 years and could be adopted in a population based setting.

Aims:

- To stratify adults with intermediate cytogenetic risk AML (i.e. M1/M2) 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-

- 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 0.9-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-

- Summary/Conclusions:

- EVI1 transcript levels at diagnosis could further stratify adult iC-AML, and high EVI1 expression predicts poor outcomes in patients receiving chemotherapy only. The optimal cutoff value which best differentiates patients is different from the normal upper limit.

Grant support: The Nature Science Foundation of China (81370657, 81370639 and 81570130).

PB1678
EFFICACY AND SAFETY OF DECITABINE IN ELDERLY AML PATIENTS: A REAL LIFE MULTICENTRE EXPERIENCE OF THE NETWORK RETE EMATOLOGIA LOMBARDIA
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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 patients were treated. Decitabine was given in a 5 days-adapted dose of 9 mg/m² for 5 days every 4 weeks. 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 OF CAI/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, infection rates are a serious complication warranting intensive supportive care.
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 unsupervised 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 <60 years old, CR rates of about 55% may be achieved.

Aims: We tested in a multicenter prospective phase 2 study the efficacy and safety of clofarabine, cytarabine and mitoxantrone (CLAM) in AML patients in first relapse or refractory to first-line daunorubicin / cytarabine induction therapy.

Methods: Consecutive patients aged 18 to 65 years in first relapse or refractory to first-line dose-intensified daunorubicin / cytarabine were recruited. Bone marrow pathology and karyotype at diagnosis and relapse were centrally reviewed. Next-generation sequencing of a myeloid panel of 67 genes was performed. Re-induction CLAM comprised clofarabine (40mg/m2/day, days 1-5) and mitoxantrone (12mg/m2/day, days 1-5). Bone marrow assessment was done on day 28 using standard criteria. Treatment toxicity was evaluated using the Eastern Cooperative Oncology Group Common Toxicity Criteria (ECOG-CTC). Survival were determined using Kaplan Meier method. The primary outcome was the response on day 28. Secondary outcomes were treatment toxicity, leukaemia-free and overall survivals.

Results: In this interim analysis, 24 patients (14 men, 10 women) with a median age of 44.5 (19-66) years were treated. Karyotypic and genetic profiles were: normal karyotype (N=8) (NPM1 mutant, N=1), FL/T3-ITD, N=8), t(8;21)(q22;22)(N=4) (KIT D816V mutant, N=1), inv(16)(p13.2;p22)(N=16), trisomy 13 (N=1), near-triploidy (N=1), and complex karyotype (N=1). Twenty patients (83.3%) responded (CR, N=16; CR with incomplete hematopoietic recovery, N=4). Eight responding patients underwent allogeneic haematopoietic stem cell transplantation. Grade 3/4 haematologicoxic events were observed in 17 (70.8%) and 2 (8.3%) patients respectively. Grade 1/2 rash was observed in 4 patients (20%). Cardiotoxicity or treatment-related mortality was not seen. With a median of follow-up of 4 (1-32) months, 6 patients relapsed. The 12-month overall and leukaemia-free survivals were 81.7% and 66.8% respectively.

Summary/Conclusions: CLAM resulted in a high CR rate for AML in first relapse or refractory to first-line induction therapy, which was associated with an acceptable toxicity profile.

PB1680
FATAL EVOLUTION IN THE FIRST 96 HOURS OF PATIENTS DIAGNOSED WITH ACUTE LEUKEMIA: ANALYSIS OF A SERIES OF 346 CONSECUTIVE CASES OF ACUTE LEUKEMIA
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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 leukemia who died within the first 96 hours after diagnosis in our centre in the last 15 years.

Methods: We studied all cases of acute leukemia 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 leukemia 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 leukemia (AML, 64%) and 124 of acute lymphoblastic leukemia (ALL, 36%). Thirty-three patients were diagnosed of acute promyelocytic leukemia (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 of life (range 21-96). The distribution of clinical and analytical findings 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 2/7 patients, and severe thrombocytopenia (Plt ≤ 20 x 10^9/L) in 3/7. There was possibility of severe 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 leukemia 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 predominate 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 Leukemia - 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 (18-73); and there was no significant gender difference (38 females vs 44 male [46% vs 54%]). All patients had active disease, 78 (74.3%) of them received 3+ (idarubicine - 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 9.9 (3-14) days in patients receiving prophylaxis and 12.7 days (1-68) in prophylaxis discontinuations 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 (7±1). Discontinuation was due to adverse events in 6 cycles (5.7%), and due to other reasons (diabetes, 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 galactomannan 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 prophylaxis (13/20; 65.0%) (p<0.0024). 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 benefit in patients receiving posaconazole prophylaxis. 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 LEUKEMIA 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 as consolidation in FLT3 ITD+ patients, but this way could be hard in frail and old patients with a low access to transplant techniques. On the other hand, the development of target drug therapy – FLT3-kinase inhibitors gives us a new hope for improvement in the treatment results of such poor-prognosis subset of AML patients.

Aims: To assess the frequency of FLT3 gene mutations and its impact on clinical course and survival of the patients with acute myeloid leukemia (AML) in routine clinical practice.

Methods: We have analyzed FLT3 gene mutation frequencies, complete blood count (CBC) parameters, karyotype and survival outcomes per FLT3-mutation status in 199 patients with AML (83 male / 116 female). The median age at diagnosis was 52 years (20-86 years). To determine FLT3 gene mutations we observed next FLT3 gene mutations rates: FLT3-ITD - 22.6%, FLT3-TKD - 6.5% and FLT3-ITD+TKD - 2.6%. We confirmed the role of FLT3 gene mutations as an unfavorable factor for AML patients in routine clinical practice by own experience.

Figure 1.

Summary/Conclusions: We confirmed the role of FLT3 gene mutations as an unfavorable factor for AML patients in routine clinical practice by own 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 LEUKEMIA 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 cardiovascular toxicity risk and promoting vascular complications. Patients with co-morbid ischemic heart disease (IHD) 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 co-exacerbator of 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 44 (66.7%) males and 22 (33.3%) females. The median age was 57 years (26–72 years), ECOG 0–1. The duration of IHD 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 completion. To determine the clinical failure rate we used the method of polymerase chain reaction (PCR) with subsequent restriction. FLT3 gene mutations were classified as internal tandem duplication (FLT3-ITD) and point mutation in the “A-loop” (FLT3-TKD). Statistical analysis was included Kruskal-Wallis ANOVA and Kaplan-Meyer curves.

Results: We observed next FLT3 gene mutations rates: FLT3-ITD - 22.6% (45/199), FLT3-TKD 5.5% (11/199), FLT3-ITD and FLT3-TKD in combination 1.0% (2/199), other 70.8% (141/199) patients had no mutations (FLT3-). CBC data at the time of diagnosis were as follows (median [max-min]): - FLT3-TKD: Hb 9.7 (3.7-13.0) g/dl, WBC 40.3 (0.6-400.0) x 10⁹/l, blasts 80% (21-100), platelets 60 (2-140) x 10⁹/l; - FLT3-TKD: Hb 10.2 (5.8-12.8) g/dl, WBC 62.4 (1.7-362.0) x 10⁹/l, blasts 68% (23-100), platelets 55 (12-115) x 10⁹/l; - FLT3-ITD+TKD: Hb 5.8, 8.4 g/dl, WBC 37.0, 157.0 x 10⁹/l, blasts 65%, 86%, platelets 38, 186 x 10⁹/l; - FLT3- Hb 9.0 (2.8-14.0) g/dl, WBC 12.9 (1.0-260.0) x 10⁹/l, blasts 64% (20-103), platelets 63 (1-334) x 10⁹/l; Significant differences across the groups were seen only in W5.5 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 (7±3, 5±2, HAM). Transplantation of hematopoietic stem cells (SCT) was performed in 28 (allo/autot 17/11) (14%) patients: FLT3-ITD allo-3; FLT3-TKD allo-1, auto-1; FLT3-allo-13, auto-10. We found significant (p=0.00024) differences regarding to OS between FLT3-ITD, FLT3-TKD and FLT3- patients (Figure 1). Median survival times were: 5.1 months for FLT3-ITD, 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 own 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 [NO₂−] 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 [NO₂−] 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 [NO₂−] 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, 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 vasodilation, 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 vasodilation, thus reducing the risk of early anthracycline cardiotoxicity development.

Methods: Clinical data from newly diagnosed M6-AML patients between 2002 and 2012 at 11 academic centers were retrieved from the electronic registry data of AML/MDS working party of Korean Society of Hematology. Conventional cytogenetic analysis was performed on metaphase cells prepared from bone marrow aspirate by G-banding technique. Patients were classified according to the UK MRC cytogenetic risk criteria and the International Prognostic Scoring System (IPSS) risk groups for MDS based on karyotypes. Survival curves were analyzed using the Kaplan-Meier method and compared with a log-rank test. A p-value <0.05 was considered statistically significant.

Results: A total of 84 patients with AEL (M6-AML) as defined by 2008 WHO classification criteria were included in this study. The median age at diagnosis was 55 years with following distribution: age ≤ 49, 34 patients (40.5%); age 50 – 59, 17 (20.2%) patients; 60 – 69, 19 (22.6%) patients; age ≥70, 14 (16.7%) patients. There were 50 (59.5%) males and 34 (40.5%) females. Median hemoglobin, white blood cell count, and platelet count were 8 g/dL, 3.69 × 10⁹/L, and 58 × 10⁹/L, respectively. Peripheral blood blasts were observed in 55 (65.5%) patients. Cytogenetic risk 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. Four (5.0%) patients had t(9;22)(q34;q11).2). Cytogenetic risk group assignment 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 allogeneic 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 overall 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).
clinical condition that limits the possibility to conduct large prospective clinical studies. All publications present small retrospective data and case reports. Most of them conclude that pregnancy doesn’t affect the prognosis of acute leukemia.

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 the Russian Acute Leukemia study group has treated 33 with de novo AML, pregnant women (Me-27 (21-42) yrs), AML was diagnosed in the 1st trimester in 1 woman (3%), in the IIInd-15 (45,5%), 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=5), -7/(-7q) (n=4), translocations involving gene MLL (n=2), 1 pt - inv3/7 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 was started at 23 (14-32)nd weeks of gestation. Classical 7+3 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 treatment study-protocol.

Results: As our data show, AML in pregnancy is characterized by high prevalence of unfavorable cytogenetic abnormalities (46%), that is substantially different from AML in non-pregnant women of the same age (11,5%) (p=0,006) [Blood 2016,128,22.p5171]. 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 reached in 100% (9/9) from the intermediate and in 80,0% (8/10) from the poor prognostic group. Primarity resistance was registered in 6/30 pts (20%). Antenatal fetal mortality was registered in 2 cases at the 21ndand 32nd weeks during induction. 29 children were born. Allogeneic 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 10y 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 combination with 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 21st March 2016 we treated 67 patients with a regimen including clofarabine at 22.5 mg/m<br>² daily on days 1-5, followed after three hours by cytarabine at 1 g/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, we noted significant delay in the hematologic 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% (24,4%) 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 leukaemia (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 analyse. 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 for patients aged ≥65, with a high 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 AML patients.

Summary/Conclusions: Irrespective of treatment intensity, patient age, performance status and the presence of comorbidities are the top three drivers of treatment selection for physicians. In addition to patient age, identification of the other key drivers for therapy selection and the physician awareness of them is critical to ensure patients receive the most appropriate therapy. This improved awareness could also lead to better communication tools for patients and improve shared decision-making.

Table 1. Top 5 patient characteristics considered by physicians when choosing high- or low-intensity treatment in AML.

<table>
<thead>
<tr>
<th>Top 5 drivers of selection</th>
<th>Total Physicians (N=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-intensity chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Patients aged &lt;65 years</td>
<td>41 (67%)</td>
</tr>
<tr>
<td>Good performance status (ECOG score 0-1)</td>
<td>39 (64%)</td>
</tr>
<tr>
<td>Patients without comorbidities</td>
<td>37 (61%)</td>
</tr>
<tr>
<td>Patients eligible for stem cell transplant</td>
<td>33 (54%)</td>
</tr>
<tr>
<td>Patients with mutation in the CEBPA gene</td>
<td>33 (54%)</td>
</tr>
<tr>
<td>Low-intensity chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Patients aged &lt;65 years</td>
<td>35 (57%)</td>
</tr>
<tr>
<td>Very poor or poor performance status (ECOG score 2-3)</td>
<td>36 (62%)</td>
</tr>
<tr>
<td>Patients with comorbidities</td>
<td>36 (62%)</td>
</tr>
<tr>
<td>Patients ineligible for stem cell transplant</td>
<td>23 (38%)</td>
</tr>
<tr>
<td>Patients with prior cancers / previous to radiation therapy or chemotherapy</td>
<td>23 (38%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Irrespective of treatment intensity, patient age, performance status and the presence of comorbidities are the top three drivers of treatment selection for physicians. In addition to patient age, identification of the other key drivers for therapy selection and the physician awareness of them is critical to ensure patients receive the most appropriate therapy. This improved awareness could also lead to better communication tools for patients and improve shared decision-making.

PB1688

IRAN LONG NON CODING RNA ARE DOWN-REGULATED IN POOR PROGNOSIS AML PATIENTS

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Background: IRAIN which is produced from the insulin-like growth factor type 1 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 migration, 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 ITD 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 prognostic group (t(8;21) patients, Wilcoxon rank test, p=0.008).

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.

Results: 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 and phosphorylation of FLT3. FLT3 TKD mutations are mediated 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 Invisioctebes® 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 lines, 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 amplings were analyzed via capillary electrophoresis. The assay measured the ratio of signals from mutation against a background control sample. A FLT3 mutation was detected (and reported as positive) if the mutant/WT type SR met 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 cutoff 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 Invisioctebes® LeukoStrat® CDX FLT3 Mutation Assay is an internationally standardized assay.
the mouse or keypad. The software utilises the latest strides made in web technologies to respond to the varying screen sizes of devices, and display suitably sized graphs and gating information accordingly. Collaboration between parties is facilitated - a lab technician running the sample can upload the sample and instantly share it with other parties with the required permissions. Analysis, such as gating, can take place immediately and can then be instantly shared via a web URL. No sensitive file data is displayed within the platform. All data transfer happens via SSL encryption.

Web app is available at https://www.redmatterapp.com

Figure 1.

Summary/Conclusions: The latest web technologies can be effectively harnessed to enhance flow cytometry analysis and allow for faster, more accessible and more collaborative analysis. Within the field of haematology in particular, this opens up the option of remote diagnosis - a haematologist need not be in the lab, or even in the same country, to deliver a diagnosis.

PB1692
FLAG-IDA IN THE TREATMENT OF ACUTE LEUKEMIA: SINGLE-CENTER EXPERIENCE
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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 groups usually use the FLAG-IDA protocol (Fludarabine 30 mg/m2 days 1-4, Idarubicine 12 mg/m2 days 1-3, ara-C 2 mg/m2 days 1-5) refractory AML. Based on European Risk Score (ERS) (ERS) 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.

Figure 1.

Results: 65 patients received treatment with FLAG-IDA protocol between 2007-2016, 36 of them female, with 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 (23% of patients) 38% (n=15) refractory AML. Based on European Risk Score (ERS) 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 results of our followed 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).

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, skin was involved in 50% of patients, 1 pt presented with a leukemic picture. Biopsies revealed diffuse, monomorphic infiltrate of medium-sized blast cells with irregular nuclei, fine chromatin and dense cytoplasmic granules. Immunohistochemical analysis showed strong positivity for 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.

PB1694
PREDICTIVE RELEVANCE OF CLINICAL CHARACTERISTICS IN PEDIATRIC PATIENTS WITH RELAPSED ACUTE MYELOID LEUKEMIA TREATED AT SINGLE INSTITUTION– REPORT OF AN OUTCOME ANALYSIS
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Background: Most AML patients ultimately die from their disease. In our case series 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.
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 1:1.2 and median age at diagnosis 5.5 years (Min: 1.3 months, Max: 13.8 years). 12 patients were excluded from further analysis for not being able to complete induction therapy. Among the remaining 143 patients (10 of 143) had concomitant malignancies. 85.7% (120 of 140) were CNS-1, 27.4% (20 of 73) had MLL Gene rearrangement, 21.2% (14 of 66) were positive for TEL AML/RUNX1/RUNX1T1 and 22% (13 of 59) had PML/RAR (+). Trisomy 4, 10 or 17 was not seen among any of 13 patients tested. Most commonly observed FAB classification was M5 (23.5%, 24 of 102) followed by M2 (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: 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 was 38.8% (52 of 134). Most common site of relapse was bone marrow (75%, 39 of 52), PML/RAR (P-Value: 0.044), Post-Induction BM Classification M-3 (P-Value: 0.034) and AML High Risk (P-Value: 0.003) were found to be significantly associated with Relapse. Age at diagnosis, or Time to CR-1 were not found to have any association with relapse. 51.9% (27 of 52) who relapsed, went for HSCT. With a median follow-up of 68.8 months, five year overall survival for our cohort of patients was (0.567±0.046); significantly poor (P-Value: 0.001) in relapsed (n=52, 0.179±0.051) compared to non-relapsed (n=82, 0.865±0.041); resulting in a five year overall survival of 0.47±0.044. Among relapsed group (n=52), five year overall survival was significantly better (0.160±0.073) for those who received HSCT (27) than who did not (n=25, 0.114±0.073; P-Value: 0.029). Five year overall survival was also significantly better for Non-Relapse group (n=31, 0.828±0.070) compared to relapsed patients (n=27, 0.160±0.073; P-Value: 0.003) who was administered (n=58).

Summary/Conclusions: 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.

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 described in mouse model as increasing chemoresistance, invasion and laboratory features allowing to make the diagnosis of AML. No evidence of a preceding CML (splenomegaly or basophilia) was found. The karyotype and laboratory features allowing to make the diagnosis of AML. The breakpoint in 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)/dbl-like 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 used.

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 (splenomegaly or basophilia) was found. The karyotype and laboratory features allowing to make the diagnosis of AML. The breakpoint in 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)/dbl-like domain, completely absent in p190 proteins. This truncation could increase the BCR/ABL oncogenic activity.

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consolidation chemotherapy was postponed, relapsing without reach the already planned bone marrow transplantation. At the bone marrow 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 p190 e6a2.

Figure 1.

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

HYPOMETILATING AGENTS AS SALVAGE THERAPY IN RELAPSED OR REFRACTORY 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(8;21), 7 were AML MRC, 1 was therapy-related AML, 6 were AML NOS. Two patients were favorable risk sec ELN 2010, 11 were intermediate I and II and 2 were adverse risk. Seven patients were treated with decitabine and 8 with azacitidine. Five patients reached CR or CRi after HMA. All 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 16; median number of hospitalization days during HMA therapy was 16; median number of hospitalization days during HMA therapy was 16; median number of hospitalization days during HMA therapy was 16; median number of hospitalization days during HMA therapy was 16; median number of hospitalization days during HMA therapy was 16; median number of hospitalization days during HMA therapy was 16. Sixty seven days from the starting of HMA and median EFS was 70 days. Median OS in patients with refractive therapy was 91 days and median OS in relapsed patients was 331 days (p=0.0049). Median EFS in patients with refractory disease was 57 days and median EFS in patients with relapsed disease was 198 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: 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 protocol-based cytarabine therapy showed 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, tolerance, outcomes and adverse events 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 (10-75) years old presented at diagnosis with: thrombocytopenia (22), leukaemia (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=100%). Protocol 1 (AraC) was applied to 16 patients and 2 to 12 patients. Complete molecular remission was achieved after a median of 2 chemotherapies (1-3). Efficacy could not be compared between protocols because there was only 1 relapse in Protocol 2, refractory to chemotherapy, ATRA, arsenic trioxide and allogeneic transplantation. However, there were significant differences in tolerability and toxicity. Patients in Protocol 1 had significantly higher transfusion needs compared to Protocol 2 (p<0.001): 9(2-15) versus 1(0-17) red blood cell and 11(3- 32) versus 2(0-10) platelet transfusion. Duration of grade 4 leukaemia was significantly higher in Protocol 1 [16(5-19) versus 9(0-18) days, p=0.002]. The same was true for neutropenia (p=0.04) and resulted to higher infection rates in Protocol 1 (58% versus 17%, p=0.03), including 2 aspergillosis and 1 fatal sepsis. 10-year overall survival probability was 73.1%, with no difference between Protocols.

Summary/Conclusions: Our study confirms that early mortality is a significant issue in APL, in particular for older patients. AraC can be safely omitted from treatment of low- and intermediate-risk patients, resulting in significantly reduced toxicity.

PB1699

DISEASE CHARACTERISTICS AND TREATMENT PATTERNS OF AML PATIENTS <60 YEARS OLD VERSUS ≥60 YEARS OLD

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Background: There is limited real-world data in patients with acute myeloid leukemia (AML) that looks at presenting disease characteristics and subsequent treatment decisions made for patients <60 and ≥60 years of age in the United States (US).

Aims: This analysis examined the characteristics of patients <60 years of age and ≥60 years of age at the point of AML diagnosis and further investigated subsequent treatments.

Methods: Data from the Adelphi AML Disease Specific Programme, a real-world, cross-sectional survey conducted between February–May 2015, were analyzed. A total of 61 hematologist/oncologists provided data on their 457 AML patients treated at various stages of AML. Disease characteristics upon
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 reassigned 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 old. 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 60% (n=98) of patients ≥60 years of age (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 cytarine-based regimen and the most common low intensity treatments were low dose cytarine-, 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 characteristic</th>
<th>&lt;60 years old</th>
<th>≥60 years old</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>0.16</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>Anticoagulant</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>No symptoms at diagnosis</td>
<td>Frequent symptoms (SDI)</td>
<td>0.01</td>
</tr>
<tr>
<td>Performance status</td>
<td>CECO score at diagnosis = 0</td>
<td>Fully active, able to carry on all pre-disease performance without restriction</td>
<td>0.001</td>
</tr>
<tr>
<td>Diagnostic tests</td>
<td>No. of tests used to establish AML diagnosis (median SDI)</td>
<td>5.5 (3.6)</td>
<td>4.7 (3.6)</td>
</tr>
<tr>
<td>Physician-defined prognostic category</td>
<td>Favorable</td>
<td>Intermediate</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>100 (17%)</td>
<td>84 (20%)</td>
<td>22 (9%)</td>
</tr>
<tr>
<td>Presence of leukocytosis</td>
<td>25%</td>
<td>90%</td>
<td>9/10 (90%)</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 more effective high intensity treatments currently available to treat AML.

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 myeloid leukemias due to the usage of ATRA in the treatment standard with chemotherapy. 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 of t(15;17) or PML/RARA. Induction therapy was carried out according to the protocol t(15;17) 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 16.5% (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%, TP53 mutations in FLT3-ITD and NPM1 in 7.5%(7), 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. The signs of presence of leukocytosis had the patients with the combination of FLT3-ITD and NPM1 mutations. There were the patients with high leucocytosis, presence of CD56 and CD2 immunophenotypic markers, who didn't achieve remission or had the recurrence when the treatment was dropped. The presence of leucocytosis was detected in 25% of cases, in 90% (9/10) of cases leucocytosis was combined with FLT3-ITD mutations and 80% of these patients subsequently had the recurrence. Within the patients with the combination of FLT3-ITD and NPM1 mutations who brought into remission after the first course of chemotherapy these mutations were not detected later on. There were the patients who had leucocytosis rate less than 20x10 9/l and didn’t have CD56 and CD2(11.5%) at the time of verification. The presence of TP53 mutation was combined with high leucocytosis of the patient and with the absence of effect on the conducted therapy. When analyzing the immunophenotypic markers CD56 and CD2, they were detected in 75% of the patients, but in the absence of gene mutations and leucocytosis, such patients had a favorable prognosis (16.7%(13/80, p=0.046)).

Summary/Conclusions: 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.
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 aorticole 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 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, 2.41×10^6/L, with 4% blasts, 26% segmented neutrophils, 3% band neutrophils, 39% lymphocytes, and 26% monocytes. Bone marrow aspirations revealed hypercellularity with blasts, which were mast cells with medium cell size, oval-arower shape vesicular nuclei, fine chromatin 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 CD3, 30%; CD4, 7%; CD5, 9%; CD10, 10%; CD11b, 30%; CD14, 20%; CD15, 20%; CD19, 20%; CD20, 20%; CD31, 10%; and CD45, 6% and TdT). Immunofluorescence study revealed the presence of minor BCR-ABL1 (e1a2) fusion transcripts. Chromosome analysis of bone marrow cells failed because of insufficient mitotic cells. Immunogolden heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirations.

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 hematopoietic 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 gene has not been described in the literature for patients with tAL with mixed phenotype, after hematologic malignancy. According to the WHO 2016 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 alkylating agent and topoisomerase II inhibitor therapy for DLBCL.

PB1704
CLINICAL, CYTOMORPHOLOGIC AND IMMUNOPHENOTYPIC/IMMUNOHISTOCHEMICAL 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 hematological 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 leukaemic. 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). In the blood count, average concentration of Hb was 108g/l (range 87-154); WBC 6,38x10^e9/l (range 2,6-12); Plt 147,8x10^e9/l (range 20-282). Hemorrhagic diathesis was registered in 3/8; splenomegaly in 6/8 (average diameter by ultrasound exam 140mm, 110-150mm); and hepatomegaly existed in 3/8 pts (average diameter 166mm, 140-200mm). Cutaneous infiltrations were present 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, immunocytochemistry 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 indented vesicular nuclei, fine chromatin 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 CD3, 30%; CD4, 7%; CD5, 9%; CD10, 10%; CD11b, 30%; CD14, 20%; CD15, 20%; CD19, 20%; CD20, 20%; CD31, 10%; and CD45, 6% and TdT). Immunofluorescence study revealed the presence of minor BCR-ABL1 (e1a2) fusion transcripts. Chromosome analysis of bone marrow cells failed because of insufficient mitotic cells. Immunogolden heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirations.

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 lethal 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.
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 methylprednisolone 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/hr (95% CI: 0.196 – 0.204) and liver and lung clearance was used. Defining clearance of 0.278 (95% CI: 0.181 – 0.390) 1/day, corresponding to target-mediated drug disposition of rituximab was recognized to best 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, whereas in patients with disease progression it was 82.2% 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 INFILTRATION IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA
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Background: Bone marrow infiltration (BMI) evaluation plays a key role in lymphoma staging, treatment and prognosis. The role of PET/CT in the assessment of BMI is still controversial, especially in non-Hodgkin’s lymphoma (NHL).

Aims: To evaluate the role of 18F-FDG PET/TC in bone marrow infiltration for the diagnosis of Non Hodgkin Lymphoma. We compared 18F-18FDG PET/TC visual and quantitative analyses with bone marrow biopsy in NHL patients.

Methods: Fifty patients with newly diagnosed NHL from February 2011 to February 2016 were retrospectively analyzed. Of these, 26 (group A) patients had aggressive NHL and 24 (group B) indolent NHL. To detect BMI on the posterior iliac crest, two different evaluation methods were used: 1) maximum standardized uptake values (SUVmax) and 2) maximal standardized uptake values (SUVmax, cut-off >2.5), and 3) Deauville score (categorical). Each method was applied in the whole patients cohort, in group A and in group B. Images were blindly reviewed separately by 3 nuclear medicine physicians. PET/CT results were compared with the bone marrow biopsy performed after imaging in all patients. Deauville score was used to evaluate the increment in net benefit (NB) obtained considering the Deauville score over a biopsy-all strategy.

Results: The prevalence of a positive biopsy was 38% in whole cohort, 19% in group A and 58% in group B. In the whole cohort, sensitivity, specificity and accuracy were 56%, 56%, and 58%, respectively. For group A, specificity and accuracy were 60%, 78%, 55% and 57% and for SUVmax; 47%, 81% and 68% for Deauville score. In group A, sensitivity, specificity and accuracy 76%, 69% and 72%, respectively. For visual analysis; 40%, 52% and 50% for SUVmax and 20%, 71% and 62% for Deauville score. In group B, sensitivity, specificity and accuracy were 100%, 100% and 58% for visual analysis; 64%, 68% and 52% for SUVmax; and 57%, 100% and 75% for Deauville score. At probability threshold equal to the prevalence of a positive biopsy, the increase in NB by Deauville score was 0.11 in the whole cohort, 0.02 in group A and 0.33 in group B. In this latter group, biopsying patients on the basis of the Deauville score is a strategy that reduced the biopsy rate by 24%. Without missing any BMI.

Summary/Conclusions: FDG-PET/TC visual analysis has a limited value for detecting BMI in patients with NHL, while quantitative analysis by Deauville score provides a higher diagnostic performance. Noteworthy, the high positive predictive value in patients with indolent NHL suggests a potential role of FDG-PET/TC in avoiding bone marrow biopsy in this subtype of lymphoma.

PB1708
LOW ALBUMIN LEVEL CORRELATES WITH POORER SURVIVAL OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: SERBIAN LYMPHOMA GROUP EXPERIENCE
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Background: Current prognostic scores are not sufficient to define high risk patients with diffuse large B cell lymphoma (DLBCL). Besides parameters included in the International Prognostic Index (IPI), other clinical and laboratory parameters can be evaluated as potential prognostic markers. However, contradictory data have been reported.

Aims: The aim of this study was to evaluate prognostic significance of clinical and laboratory parameters on the overall survival (OS) of patients with DLBCL.

Methods: A total of 393 patients (188 females/205 males) with the median age of 56 years (range 18-87) were included. All patients were initially treated with rituximab plus CHOP (Cyclophosphamide, Doxorubicine, Vincristine, Prednisone) or CHOP-like protocols.

Results: Ann Arbor stage I, II, III and IV had 56 patients (14.2%), 142 (36.1%), 71 (18.1%) and 124 (31.6%), respectively. Bulky disease had 99 patients (25.2%), B symptoms 263 patients (66.9%), and poor performance status according to the European Cooperative Oncology Group (ECOG) ≥2 had 82 (20.9%). 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 lymphoma size was 3.85 cm (range 0.15-150 cm). Absolute monocyte count (AMC) was 0.64x10⁹ (range 0.07-8.5x10⁹/L). Low AMC was recognized to best describe the data. The nonspecific 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, whereas in patients with disease progression it was 82.2% 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.
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). Treatment patterns and response to treatment were captured using a treatment group. Possible remission was defined as no additional chemotherapy and no supportive care use or receipt of supportive care <30 days after end of line of therapy (LOT) for <30 days. Lack of remission was defined as receipt of supportive care ≥30 days after end of LOT for ≥30 days. Progression was defined as initiation of another LOT or evidence of supportive care ≥30 days after end of a LOT.

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. Of 33 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, with 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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1National Research Center for Hematology, 2Federal State Institution “Russian Hematology Ministry of Health Russian Federation diagnosis of high grade B-lymphoma were established in 47 patients: 13 – double hit, 34 – not otherwise specified. We had available biologic samples from 32 pts with HGBL: 11 pts with WT-TP53, 9 pts with c.MUT-TP53 and 12 pts with c.TP53 rearrangement. (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, TP53-TP35 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; 16%, 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
THIOTEPA BUSULFAN CYCLOPHOSPHAMIDE, A TOXIC CONDITIONING FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CENTRAL NERVOUS SYSTEM LYMPHOMA: REMISSION OR INFECTION
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PB1714

TREATMENT RESULTS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA FROM HIGH RISK AND HIGH-INTERMEDIATE RISK GROUPS
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Background: Using of Rituximab-containing regimens, as the «gold standard» lines of chemotherapy (with high doses Methotrexate in first or second line) could be considered as the most efficient treatment regimen in patients with DLBCL from high and high-intermediate risk groups.

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 CHIMOS® software. TBC combined Thiotepa (250mg/m²/d from d-9 to d-7) Buflusam (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/PT and transplant related mortality (TRM) (defined by death occurred 3 months after ASC/PT). The level of ORR and CRR was significantly higher (81.4%, p=0.004). However, the rates of anemia, thrombocytopenia and hepatotoxicity were comparable in three groups. Neutropenia, febrile neutropenia and cardiotoxicity were less common and neurotoxicity was more frequent in this group (p=0.043).

Summary/Conclusions: Ours is the first prospective cohort concerning TBC/ASCT in CNSL. It shows that an important rate of CR (100% with 66% grade 3) and a TRM=21%. Neurological adverse events (37%; 9 patients with 4 comas) and infections (100% with 41% grade 3) were predominant. We documented 2 CMV reactivations and 5 fungal infections (3 candida, 1 aspergillus and 1 cryptococcus). We observed 5 deaths (4/5 older than 60 years) in first 3 months due to a septic choc, 4 associated with a persistent coma and 2 with an acute respiratory distress syndrome.

PB1715

PROGNOSTIC MODEL WITH NEUTROPHIL-LYMPHOCYTE RATIO AND PERFORMANCE STATUS IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP
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Background: Growing evidences suggest the close relationship between inflammation, host immunity, and tumor cells. The neutrophil to lymphocyte ratio (NLR) has been known to predict the prognosis in patients with diffuse large B-cell lymphoma (DLBCL).

Aims: This study was planned to confirm the prognostic and predictive value of NLR and to make a model to predict the prognosis more precisely in patients with DLBCL.

Methods: Data of 192 DLBCL patients treated with R-CHOP from 2004 to 2016 were retrospectively assessed. Patients with NLR ≥4 and <4 were determined as the high and low NLR groups, respectively. Treatment response and survival were compared according to the NLR status and using the model including NLR and other variable interacting with NLR.

Results: High NLR group was associated with old age, poor performance status (PS), elevated lactate dehydrogenase, and more advanced prognostic indices than low NLR group. High NLR group had a low complete response (CR) rate compared to low NLR group (57.5% vs 81.4%, p=0.004). However, the hazard ratios for CR as prognostic NLR were not significantly associated in multivariate analysis, which showed strong interaction between NLR and PS. The model composed of NLR and PS could stratify the patients into low-, intermediate-, and high-risk groups for overall survival (OS). On multivariate analysis, compared to low risk group, the hazard ratios of intermediate and high risk groups on OS were 1.871 (p=0.019) and 2.733 (p=0.004).

Summary/Conclusions: High NLR is associated with poor treatment response and unfavorable clinical features in DLBCL. The prognostic model using NLR and PS can predict more precisely the prognosis of this population and needs to be validated in the independent cohort.
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) and poorer PS (≥2). Eight patients were alive at the time of the analysis, with a median follow-up of 55 months (range: 2–136 months). The 2-year OS among all patients was 25.1% (95% CI: 13.6–38.5), and the high sIL-2R group had significantly poorer 2-year OS (10.9%, 95% CI: 2.8–25.4 vs 50.0%, 95% CI: 24.5–71.0, P < 0.001). A multivariate analysis was performed using the following factors: serum sIL-2R levels (high vs low), secondary IPI (≥2 vs ????????) (Figure 1).

Figure 1. Overall survival according to serum sIL-2R levels.

Summary/Conclusions: Serum sIL-2R levels are a useful predictor of prognosis in cases of relapsed/refractory PTCL-NOS, especially among patients with low secondary IPI risk.

PB1717

AUTOIMMUNE DISEASES ARE NOT ASSOCIATED WITH INFERIOR PROGNOSIS IN PATIENTS WITH SECONDARY IMMUNODEFICIENCY SYNDROME (SIDS) – A PROSPECTIVE STUDY OF AUTOIMMUNE DISEASES AMONG PATIENTS WITH SIDS

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Background: Previous epidemiological studies have shown that autoimmune diseases increase the risk of lymphoma development. Immune dysregulation could be the possible underlying pathogenesis. Whether autoimmune diseases deteriorate outcome of lymphoma patients, however, remains unclear.

Aims: The objective of this study is to compare the clinical outcome among lymphoma patients with and without autoimmune diseases.

Methods: From January 2008 to November 2016, we retrospectively reviewed medical records of 913 newly diagnosed lymphoma patients. From these 913 lymphoma patients, 34 (3.71%) patients were diagnosed to have autoimmune diseases before their lymphoma identification. Among these 34 patients, six lymphoma patients (11/34; 32.3%). The complete remission rate for lymphoma patients with and without autoimmune diseases were 72.0% and 83.3%, respectively (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: 0.59–2.96, P = 0.627).

Summary/Conclusions: The results of this case-control study showed the autoimmune disease was not a poor prognostic factor for lymphoma 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 (JCO. 2006;24:5711). 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). Neither of the two systems has been validated 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. 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 intensity 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) Analyze the impact of CIRS score in OS; 3) Analyze the impact of GCSF prophylaxis on neutropenia.

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 question 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 1-16) 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 18 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).
Efficacy and Safety of Ibrutinib in Relapsed/Refractory Mantle Cell Lymphoma in Real-Life—A Multicentric Study (R.E.P.-Apulian Hematologic Network)

Aims: To investigate the clinical use of ibrutinib as a single-agent in 31 patients with relapsed or refractory mantle cell lymphoma (MCL) to obtain additional information about predictive factors, outcomes and toxicity in a real-life context.

Methods: We studied a group of 31 patients (52% treated or still in treatment) with MCL 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 (MIP1, MIP2, 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: At the initiation of ibrutinib therapy, the median age was 70 years (range, 45-82), 100% of patients had high risk MCL according to the MIPI score, 83.9% of patients had disease stage III or higher, 41.9% of patients had bone marrow involvement, and 45.2% of patients presented extranodal involvement of MCL. 26 pts were treated for relapsed MCL, 5 for refractory disease. They received a median of 2 (range, 1-5) prior regimens including different chemo-immunotherapeutic schemes, ASCET and newer agents such as bortezomib, lenalidomide and/or etanercept. The most common AEs were fatigue (13% of pts) and weight increase (13% of pts), followed by diarrhea and bleeding (grade ≤ 2) (6.4% of pts), respectively.

Summary/Conclusions: Single-agent ibrutinib therapy shows a high response rate and produces rapid responses regardless of the number and quality of previous regimens. However, the quality of time and rate of response does not seem to be predictive of 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 patients suggests that ibrutinib may have an anabolic effect, including alterations in body mass and fat distribution.

PB1723 Hematological Malignancies in Solid Organ Transplant Recipients: Retrospective Single-Center Analysis in Japan

Aims: To identify prognostic factors, we analyzed gender segregation from the medical records of patients with diffuse large B-cell lymphoma (DLBCL) treated at Hokkaido University Hospital between 1965 and 2015 were reviewed retrospectively. 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 Kai-square test.

Results: A total of 16 cases of HMs were identified, 9 post-transplant lymphoproliferative disorder (PTLD) and 7 myeloid neoplasms (MNs). The incidence of PTLD in patients without disease, with PTLD and with myeloid neoplasm, respectively.

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 gender segregation awareness is necessary for a clinical approach for myeloid neoplasms following solid organ transplantation are needed.

PB1724 MYC Rearrangement Has a Strong Prognostic Impact in the Female Patients With Diffuse Large B-Cell Lymphoma

Aims: To determine the gender segregation of clinicopathological factors and prognosis in female patients with diffuse large B-cell lymphoma (DLBCL) treated at Hokkaido University Hospital between 1965 and 2015 were reviewed retrospectively. 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 transplant were collected contemporaneously from the time of transplant (T) until death or last follow-up. The patients were analyzed retrospectively. 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 Kai-square test.

Results: A total of 16 cases of HMs were identified, 9 post-transplant lymphoproliferative disorder (PTLD) and 7 myeloid neoplasms (MNs). The incidence of PTLD in patients without disease, with PTLD and with myeloid neoplasm, respectively.

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 gender segregation awareness is necessary for a clinical approach for myeloid neoplasms following solid organ transplantation are needed.
variante analysis was performed for the OS. Elevated LDH level, stage ≥3, PS ≥2, ≥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 out 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).

Figure 1. Overall survival.

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 reported in 23% of the patients, followed by large intestine and ileocecal (23 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% (9/29) of the eccentric lesions. The median length and wall thickness of the involved site were 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 concentric 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 1.017-1.116). 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, K187, disease stage, anatomical localization 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.

PB1726

DOUBLE-HIT AND TRIPLE-HIT LYMPHOMAS: TREATMENT AND CLINICAL OUTCOME IN A SINGLE INSTITUTION

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Background: Five to 15% of patients with diffuse large B cell lymphoma (DLBCL) present MYC and BCL2 and/or BCL6 rearrangements which are detected by fluorescence in situ hybridization (FISH) or standard cytogenetic. This rearrangement defines a subgroup of DBLCL so-called double hit or triple hit lymphomas (DHL/THL) which are included in the 2016 WHO classification revision of lymphoid neoplasm in a new category “High-grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6”. DHL/THL have an aggressive clinical course and poor response to standard chemotherapy and a median overall survival of 0.2–1.5 years. The best therapeutic option in these patients is not yet well established.

Aims: To evaluate retrospectively the incidence, clinical-biological characteristics, type of treatment, overall survival (OS) and progression-free survival (PFS) of patients diagnosed with DHL/THL and to compare them with patients with DLBCL without double/triple-hit genotype (DLBCL-noDH/TH) in a single institution.

Methods: From January 2000 to April 2016, we analyzed 18 patients with DHL/THL and 312 patients with DLBCL-noDH/TH. DHL/THL cases were identified using FISH for MYC, BCL2 and BCL6 in the tumor tissue (11 lymph node biopsy, 2 bone marrow biopsy, 1 bone marrow biopsy, 3 skin biopsy and 1 cerebrospinal fluid).

Results: The incidence of DHL/THL was 5.5%. The median age was 70 years [range 59-93]. The patients included in DHL/THL group had a higher prevalence of advanced disease and higher IPI (p=0.002). Thirteen patients received anthracyclines containing chemotherapy, 3 cito reductive treatment and 2 palliative care. No stem cell transplantation was performed in any patient as a consolidation therapy. Four out of 13 patients achieved complete remission, 3 patients partial response and 6 patients were refractory. At last follow up, 13/18 patients were dead (11 lymphoma progression, 2 infectious complications). Median follow-up 63 months. OS in DHL/THL was 9 months and in DLBCL-noDH/TH was not reached (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/TH was 5.4 and 63 months, respectively (p=0.001) (Figure 1).

Figure 1. Overall survival.
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 chemosenstive 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 consolidation with ASCT. Characteristics of those 29 patients at the time of salvage therapy were: median age 50 years (range 21-71), males 19 (65.5%), ECOG 2-4 16 (55.2%), Ann Arbor stage III-IV 23 (79.3%), B-symptoms 9 (31%), bulky disease (20.7%), extranodal involvement 20 (69%), leptomeningeal infiltration 2 (6.8%) who were treated with R-CHOP, ARAC 4 (13.8%) in patients with leptomeningeal infiltration and intensive bortuxit-like therapy 1 (3.4%) in a double hit patient. Twelve (41.4%) did not complete the full chemotherapy (8% toxicity (1 cardiac event and 1 septic shock) and 10 (34.4%) for progression. The intention-to-treat response rate was: CR 1 (3.5%), PR 4 (13.8%), refractory disease/progression 22 (75.8%) and not evaluable 2 (6.9%). Five patients underwent an ASTC (BEAM as conditioning regimen). One died during transplant due to septic shock and as progression with a median follow-up of 5 months. One patient was rescued with a third line of treatment (R-ICE) and allogeneic transplant, and is currently in CR at 7 months. Median PFS was 2 months (CI 95% 1.2-2.7) and median OS was 5 months (CI 95% 3.4-6.6). Among the 15 primary refractory patients who were treated with palliative intention, median PFS was 1 month (CI 95% 0.19-1.80) and median OS 1 month (CI 95% 0.19-2.42). Among the 317 patients treated with R-CHOP, risk factors at diagnosis for having PRD to R-CHOP were: B symptoms (HR 1.94, 95% CI: 1.05-3.61, p<0.034) and elevated LDH (HR 3.92, 95% CI: 1.61-9.51, p=0.003) (Table 1).
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 using standard CSF cytology, 8-color flow cytometry or MRI imaging. 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 CNS 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. In all, of them, were considered negative for CSF infiltration by standard cytology. Three additional patients underwent CSF flow cytometry as part of their treatment as an MRI positive for 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%. The median number of doses per patient was 6.5 (SD 1.7). CNS clearance 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: cerebellar ataxia and symptomatic neurotoxicities of neuroleptic syndromes (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 with time.

Summary/Conclusions: use of liposomal form of cytarabine for IT administration has been 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.

PB1730

RETROSPECTIVE ANALYSIS OF OUTCOMES FOR ELDERLY PATIENTS WITH STAGE 3 AND 4 DISEASE HIGH-GRADE 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 over age (1). Over 40% of patients with DLBCL are above the age of 70, and the co-morbidities 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 and Addenbrookes Hospital, Cambridge.

Results: Out 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, EOCOG 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 cycles (range: 2-5 cycles). The overall response rate was 50% (categorical assessment and 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 (4 out of 12) was 9.5 months (range: 2-42 months) and the median overall survival of living patients (8 out of 12) is at 40.5 months (range: 27-84 months).

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 pharmacogenomic targets in lymphoid neoplasm. 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 pharma-economic benefits.

PB1731

MULTIPLE NEOPLASMS CONSIST OF SOLID CANCER AND NON-HODGKIN LYMPHOMA

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Background: Malignant lymphoma is a ninth cause of death in Japan. Non-Hodgkin lymphoma(NHL) occupied more than 90%. We experienced cases and will report that we reviewed multiple neoplasms consisting non-Hodgkin lymphoma. We experienced 176 cases.

Aims: We aimed for epidemiology and prognosis improvement of malignant neoplasms including NHL. We want to look for a hint of the early detection.

Methods: We intended for multiple neoplasms 340 cases including hematological malignancy. We reviewed 190 cases of multiple neoplasms including malignant lymphoma. In 190 cases, NHL case were 176 cases. The examination factors are type of the hematological malignancy, gender, the age at onset of the first cancer, interval with the second cancer, treatment strategy. The definition of multiple neoplasms followed Warren & Gates theory. And as for the definition of synchronous and metachronous type, a diagnosis interval is less than 6 months, metachronous type interval is more than 6 months. About statistical examination, we used SPSS statistics version 21.

Results: All cases are 176 cases, consist of male 108 cases, female 68 cases, synchronous type 45 cases, metachronous type 131 cases. Double neoplasms 149 cases, triple neoplasms 25 cases, quadruple neoplasms 2 cases. The median age was 7 years (ranged 51-88years), the metachronous type was 47years (ranged 57-93years). The counterpart of malignancies, Hodgkin's lymphoma 1 case, myelodysplastic syndrome 3 cases, acute myeloid leukemia 8 cases, multiple myeloma 4 cases, gastric cancer 36 cases, colon cancer 32 cases, lung cancer 26 cases, renal cell carcinoma 6 cases, prostate cancer 12 cases, breast cancer 14 cases, urinal bladder cancer 5 cases, uterine cancer 7 cases, esophagangal cancer 9 cases, hepato-cellular carcinoma 12 cases. In double neoplasms 149 cases, metachronous type was 112 cases. The median age of first diagnosis, 68years (ranged 43-85years), the second cancer were 74years (ranged 57-89years). About interval between solid cancer and NHL, median interval time was 58months, solid cancer preceded case was 53 cases, interval was 81cases (ranged 7-564months), hematological malignancy preceded case was 59 cases interval was 55cases (ranged 8-364months). The cause of death was that 15 cases were solid cancer, 72 cases were hematological malignancy and 6 cases were accident. The median overall survival was 18months (ranged 1-211months), synchronous type 14months (ranged 2-132months), metachronous type 22months (ranged 1-116months).

Summary/Conclusions: In the case of a double cancer including solid cancer and NHL, the first cancer occurs in elderly. Diagnosis of malignant neoplasms was 3 years (4 out of 149 cases(32.2%)). The important point is that 3 years are required for careful observation at the time of malignancy diagnosis. It is necessary to discover at the early stage. So it could be a lot of treatment options formalnagous neoplasms. We think that a prognosis is improved.

PB1732

RETROSPECTIVE EVALUATION ON Efficacy and FEASIBILITY OF R-CODOX-M/IVAC REGIMEN IN AGGRESSIVE DLBCL WITH LONG-TERM USE.

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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 DHL or DPLs. From January 2011 in our centre (IRCCS AOI 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 tumor burden, DPLs, IPI score >3 or by the presence of at least 1 extra nodal 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, ifosfamide 1500 mg/sqm day 1-5, etoposide 200 mg/sqm day 1-5, cytarabine 2000 mg/sqm bid days 1-3. In both cycles CNS prophylaxis was administered. According to Ann Arbor classification, 11 patients were on stage IV, 1 on stage III, 3 in stage II 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 (69%), partial remission in 2 patients (13%). The overall response rate was 82%. Three patients (18%) were alive without disease (AWD). OS in patients with AWD was a twelve month's was 88.9% and 64.8%, respectively, not significantly lower than non DPL patients (p=n.s., median NR). 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 78.5 and 45% (median 12 months). The main toxicity during CODOX-M 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, main toxicities were the hemorrhagic cystic 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 retains a negative prognostic impact. 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 cell lymphoma is a relative rare type of diffuse large B cell lymphoma. Immunochemotherapy followed by consolidation radiation is the standard of treatment. However, the cycles of chemotherapy and the role of consolidation radiation are still under debate.

Aims: To review and analyze the treatement 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). RCHOPIP 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, 5-year survival for 4 cycles of chemotherapy and 6 cycles of chemotherapy were 88% vs 86%(p=0.42), respectively. For addition of IRRT in CR patients, 5-year survival for IFRT vs no IFRT were 83% vs 90%(p=0.93), respectively. Treatment-related mortality(TRM) was 10%(n=3) and primary refractory disease was 10%(n=3). 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. 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 is a heterogeneous disease with variable clinical characteristics. The Immunohistochemical 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 DBLCL and the expression of BCL-2, BCL-6 and MYC.

Methods: We conducted a retrospective study that included hospitalized patients with de novo CD20+ DBLCL, 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, 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/BCL-6+ in 6, and 21 patients co-expression of BCL2/BCL-6, 1 patient had MYC/BCL-2 and 1 had MYC/BCL-6. 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/BCL-6, 4 BCL-2, 2 BCL-6, 1 MYC/BCL-2, and 1 MYC/BCL-6. Of all patients who received third line treatment 3 had their line treatment switched to BFM-90 protocol (2 BCL-2, and 1 MYC/BCL-6). 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(DBLCL), 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 DBLCL in the rituximab era.

Methods: Elderly patients diagnosed with DBLCL between 2008 and 2014 were enrolled for analysis.

Results: There were 145 elderly patients with DBLCL diagnosed between 2008 and 2014. After excluding patients with primary central nervous system DBLCL (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 front line 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 (aIPI) 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 aIPI 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 DBLCL remains a challenge and complex. We co-evaluated to tailor therapeutic interventions and offer the best supportive care may reduce complications and improve the clinical outcome of these patients.

PB1737
TREATMENT OUTCOME OF MONOMORPHIC EPITHELIOBLASTIC INTESTINAL T-CELL LYMPHOMA: EXPERIENCE FROM AN ASIAN CANCER CENTER
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Background: Monomorphic epithelioblastic intestinal T-cell lymphoma (MEITL), previously type II enteropathy-associated T-cell lymphoma(EATL), primarily occurred in Asian countries. It is refractory to chemotherapy and the prognosis is poor. Intensive chemotherapy has been proposed to improve treatment outcome.

Aims: We examined the treatment outcome of MEITL in our institution.

Methods: We retrospectively searched our institutional database from 1996 to 2014 for intestinal T-cell lymphoma. Medical records were reviewed and the patients were classified on the basis of WHO-2016 classification. Patient’s characteristics, treatment modalities, response and survival were collected and analyzed.

Results: Ten patients with intestinal T-cell lymphoma were identified. One patient had enteropathy-associated T-cell lymphoma (EATL) presenting with celiac sprue. Five patients had intestinal T-cell lymphoma, NO. Four patients were diagnosed (poly)epithelioblastic epithelial intestinal T-cell lymphoma (MEITL). For patients with MEITL, median overall survival was 7.9 months (4.2-15.0 months). Median age was 46 years of age. Bowel perforation was the initial presentation in 3 patients (3/4, 75%). One patient was treated with chemotherapy with CHOP regimen, while another patient underwent surgery alone. The remaining two patients (3/4, 75%) were treated surgically followed by chemotherapy (one with CHOP, the other with BFM-90 protocol). Only one patient (1/4, 25%) entered complete response. Of concern, the unique patient achieved complete response received surgery followed by chemotherapy with Berlin-Frankfurt-Munster(BFM)-90 protocol. Remission duration was 10.3 months. He passed away 15.0 months after remission because of relapsed lymphoma.

Summary/Conclusions: Though the prognosis of MEITL is poor, operation followed by high dose chemotherapy such as BFM-90 protocol may have better treatment response, response duration and survival. It deserves further investigation.

PB1738
OSTEOPONTIN AS PRONOSTIC FACTOR OF DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma. It is a heterogeneous disease whose prognosis depends on the histological subtype (centrorganemal, non-centroorganemal), as well as other factors such as age, clinical stage, extranodal disease, ECOG scale and levels of lactate dehydrogenase (LDH) identified by established scales (IPI, NCCN-IPI). Osteopontin (OPN) a protein that is secreted by various cells and fulfills physiological functions, when produced by neoplastic cells favors tumor growth and metastasis. This has been corroborated in different types of cancer and there are few reports of cases of patients with DLBCL in which the tumor expressed osteopontin, characteristically these cases have presented an aggressive clinical behavior with extranodal disease.

Aims: To evaluate the expression of osteopontin in neoplastic lymphocytes and their association with overall survival; the percentage of patients who expressed osteopontin at diagnosis; the association between the expression of osteopontin and the histological subtype (centrorganemal, non-centroorganemal, unclassifiable); the association between osteopontin expression and age, elevation of DHL, ECOG scale, clinical stage, extranodal invasion and the application of the IPI and NCCN-IPI scales.

Methods: Tissue samples were obtained from DBLCL patients diagnosed at the Instituto Nacional de Cancerología between December 2014 and January 2016. Morphologic and immunochemistry features were studied on paraffin-embedded tissue microarray (TMA). Single antibody staining was performed for OPN. OPN expression was semiquantitatively assessed by three different pathologists scoring the proportion and intensity of stained cells. Positive cases with an expression of osteopontin in the nucleus or cytoplasm of the tumor cell. Age, ECOG, clinical stage, LDH, extranodal invasion, histological subtype, IPI and NCCN-IPI score were independently documented. Overall survival (OS) analysis was performed by the Kaplan-Meier method, the comparison between different curves was performed using the log-rank test; for the analysis of the relationship between variables we used the X2 test with a statistical significance of p<0.05.

Results: 81 patients were evaluable. 43.2% of the cases were positive for OPN in neoplastic cells. The mean survival of patients with positive OPN was 14.8 months versus 16.5 months for patients with no OPN expression (p=0.025). OPN positivity was not significantly associated with increased age, impaired functional status (ECOG 2.3.4), advanced clinical stage (III, IV), increased LDH or extranodal invasion (including central nervous system); neither was it associated with a specific histological subtype. Survival significantly decreased in patients with increased LDH (p=0.000137), ECOG 2.3.4 (p=0.000137). Survival decreased significantly as the risk measured by the IPI and NCCN-IPI scales increased (p=0.000001, p=0.000013 respectively) with an average survival of 18.6 months for the low-risk group, compared with 6.4 months for the high-risk group (Figure 1).
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.

Figure 1.

Summary/Conclusions: Our findings demonstrate that approximately half of the cases evaluated express OPN at diagnosis and tend to have a lower survival rate, however, a longer follow-up time is needed, as well as other studies that discriminate between different isoforms or post-translational modifications of osteopontin to determine if this trend can reach significance. By demonstrating OPN expression by neoplastic cells we can devise new protocols that evaluate its usefulness as a surrogate marker of tumoral activity in DLBCL using non-invasive techniques (e.g., quantification of serum levels), which would improve surveillance of these patients.

PB1739
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 RMP 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 (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.

Figure 1.

Summary/Conclusions: This single centre study demonstrated that patient selection, based upon comorbidities and performance status, for high dose combination chemotherapy in the treatment of PCNSL improves 30 day mortality, often associated with death from myelosuppression due to chemotherapy. The overall response rate, with appropriate selection of combination chemotherapeutic regimens, was improved. This also applies to patients with SCNSL in subgroup analysis. Longer follow up of patients will be needed to further demonstrate an overall survival benefit.

PB1740
AN AUDIT OF THE USE OF RASBURIACE FOR THE PREVENTION AND TREATMENT OF TUMOUR LYMPHOSIDE IN PATIENTS RECEIVING TREATMENT AT THE NORTHERN CENTRE FOR CANCER CARE, NEWCASTLE UPON TYNE, UK
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Background: Tumour Lysis Syndrome (TLS) is a known complication of haematological malignancies. Although clinical TLS is rare, the consequences are significant, with one third of affected patients requiring dialysis and an overall mortality rate of around 15%.1,2 A new British Society for Haematology (BSH) guideline was published in April 2015 to guide physicians on how to risk stratify patients based upon the Cairo Risk Stratification 20103,4, choice of prophylaxis, and treatment of established TLS5,6. We audited all patients who received rasburicase at the Northern Centre for Cancer Care from 16th April 2015 to 3rd February 2016, and compared their management with BSH guidelines4,6.

Aims: To compare our practice with BSH guidelines.

Methods: Retrospective review of electronic patient prescription records, biochemistry results, and paper notes.

Results: 27 patients received rasburicase in the study period. 20 patients met Cairo criteria/BSH criteria as having High Risk Disease (HRD) or Intermediate Risk Disease (IRD)/Low Risk Disease (LRD) with renal impairment, and therefore should have received 3mg rasburicase prophylaxis if no evidence of TLS according to the guideline. Of those 20, 11 had laboratory TLS, and therefore BSH guidelines would recommend 0.2mg/kg/day [G1] rasburicase, however only 3/11 were given the drug at treatment doses. 1/3 had clinical TLS at presentation and received treatment according to the guideline. The other 2 other patients received larger doses of rasburicase but less than the BSH would recommend. A further 7 patients with IRD received rasburicase prophylaxis but on review did not meet the criteria for rasburicase as set out in the guidelines. 5 patients died during the study period. 2 patients died on ITU of multi-organ failure <7 days into chemotherapy. A third patient died of sepsis, and the other 2 deaths were in deteriorating patients where a decision was made to palliate.

Summary/Conclusions: When assessed against BSH standards, all patients in this cohort who should have received rasburicase prophylaxis, were given the drug. 2 patients with lab TLS developed clinical TLS. 8 others with lab TLS received lower doses than the BSH would recommend, but did not progress to clinical TLS. Although there were 5 deaths in our cohort, none were directly attributable to TLS. In order to comply with the guidelines, particular importance must placed on formally assessing the TLS risk score as per Cairo guidelines.
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 HRD 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.

Aims: To evaluate the clinical impact of 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 (range26-99). 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 treat-
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 than 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 old were subjected; 12 hemophilia A patients and 7 hemophilia B patients. Factor concentrates were injected to reach the target activity of 80% 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 of 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), thromboxeblocagrost (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 49.8%. 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 nM and 376.6 nM (P=0.108, normal range, 458 nM±60). TEG index of FVIII and FIX were -1.60 and -3.77 (P=0.004, normal range, -2.24). MIN2 of CWA of FVIII and FIX were 0.62 and 0.59 (P=1.000).

Summary/Conclusions: Global hemostatic assay indicates even though IVR of FVII 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 62–75% 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 14/18 (78.3%) 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 only one had a positive family history of INH formation. Characteristics of patients is given in Table 1.

Results: INH titres prior to ITI were 1.2 - 37 (median 6.75) BU/ml. One of low responders eliminated INH spontaneously, 1 patient is waiting for ITI initiation. ITI with octocog α was initiated in 12/14 boys after 0.2 to 8.2 (median 2.0) months from INH diagnosis and completed in 9 patients. Three patients are still on ITI. INH eradication was observed in 7/9 (77.8%) of those who completed ITI. Eradication of INH was not achieved in 2 patients; both have already 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.

PB1745
APPROACH TO PREGNANCY IN NIEMANN PICK DISEASE TYPE B PATIENT
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Background: Niemann Pick Disease type A and B is a rare autosomal recessive disorder caused by sphingomyelinas deficiency resulting in sphingomyelin accumulation in macrophages of varies organs. In type B usually patients survive in adulthood. Usually, they have hepatosplenomegaly, thrombocytopenia, and dys-lipidemia. Liver and liver function are influenced, and they have bleeding risk.

Aims: Pregnancy in this situation is always risky and multidisciplinary approach is needed. Searching on Medline we found only two case reports of childbirth by women with this condition.

Methods: We presented a case of pregnancy in 34 year old women with Nie- man Pick disease type B. She had marked splenomegaly, mild thrombocytope- nia and partial respiratory insufficiency. Previously, she had two artificial abor- tions without more than expected bleeding. Also she had surgery of left side inguinal hernia and after that she was given platelet concentrates. Risk factors for pregnancy were presented to her.

Results: Laboratory controls were done periodically, ultrasonography examination of abdomen and portal vein system, lung capacity and echocardiography were performed, too. Results of CBC were stable. Repeated tests of hemostasis were normal. Hyperlipoproteinaemia type IIb with hypoHDL choledolemia was present. We assumed that platelets dysfunction could exist, therefore before pregnancy we performed platelet function tests with ADP, TRAP and collagen. All of them were below lower limit: ADP 43 (55-117), TRAP 71 (92-151), col. 30 (61-108). Ultrasonography examination of abdomen and portal vein stemi revied liver diameter 17cm, craniocaudal diameter of spleen 22cm, portal vein had not been seen. There were no sign of trombosis in portal branches. Amniocentesis was done without complications and there was no need for platelet substitution. Normal male karioity was found. We prepare her for planned caesarian section with platelet concentrates. She was given corticosteroids for lung maturation. In 35th+5 gestational week she was opera- ted. Before surgery platelets count was 87x10^9/L, she was given seven concen- trates of platelets (1 per 10 kg body weight) before and seven during proce- dure. She also received antibiotic prophylaxis. Newborn was 47cm, 2490 weight and Apgar score was 7/8. There was no major blood loss and no need for red blood cell transfusion or platelets transfusion in follow up period. We decided not to make splenectomy or partial resection because there were no significant differences in spleen measurements before and during the preg- nancy, and there was no sign of spleen trauma. Also, in literature we found data about worsening lung function after this procedure caused by more shing- myelin accumulation in pulmonal tissue. Published data and findings of our normal platelet function in our patient and experience with previous abdom- inal surgery led our decision to give her platelet concentrates before section and according to obstetrician’s estimation during the operation. Pregnancy did not cause health state deterioration in our patient and there are no clinical find- ings of Niemann Pick disease in newborn.

Summary/Conclusions: We presented a 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 assesment, platelet transfusion, splenectomy, and...
dyslipidemia treatment were 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 hæmostasis. Pregnancies 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 epistaxis, 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%. 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>
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<tr>
<td>Operation day</td>
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<td>2nd day</td>
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<td>4th day</td>
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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 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 2006. But HIRAs were not available.

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 infusion 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 limbic gap and one had mild neurologic sequela 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%) probable and obligate hemophilia carriers had high bleeding scores (≥24). 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 common 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 intracerebral hematomas and dilated cerebral ventricles. Postnatally, the diagnosis of severe congenital FVII 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 ranges 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 DEVICE

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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 antplatelets 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.

Aims: 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 implant in our hospital in 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 antplatelet 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. 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. Median 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: Acquired pure red cell aplasia associated with lymphoproliferative diseases may be a possible complication of patients on dialysis. Flow-cytometric analysis is useful for detecting a small amount of abnormal lymphocytes. Further accumulations of patients were required for understanding the pathogenesis of lymphoproliferative diseases causing acquired PRCA on dialysis.

PB1752

ADULT PATIENTS WITH ACQUIRED PURE RED CELL APLASIA: TREATED BY CYCLOSPORINE A OR CORTICOSTEROIDS - SIMILAR EFFICIENCY

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Background: Adult pure red cell aplasia (PRCA) is a syndrome characterized by a severe normocytic anemia, reticulocytopenia, and absence of erythroblasts by an otherwise normal bone marrow. Immunosuppressive therapy has been used as the initial treatment for acquired chronic PRCA. Aim: This study was conducted to compare the efficacy of cyclosporine A, and/or corticosteroids, and possible factors influencing it.

Methods: 34 cases of PRCA were retrospectively analyzed at our institution. Clinical data of 23 inpatient cases and 11 outpatient cases since 2009 October were collected. These patients were treated by cyclosporine A (CsA), and/or corticosteroids (CS), and our immunosuppressive agents if become refractory and relapsed. Results: 31 patients were evaluated in our institution (one patient lost to follow-up and two patients with short observation period). The remission induction therapy included CsA (n=13), CS (n=13), or a simultaneous combination of CsA (n=5). The initial response rate of CSa alone, CS alone, combination of CS and CsA were 69.2%, 46.2%, 80%, respectively (P=0.422). There was no statistical difference in response rate and CR rate between CsA-containing group and CS group, although the patients treated with CsA had a better response than those treated with CS (response rate 72.2% vs 46.2%, P=0.262; CR rate 33.3% vs 23.1%, P=0.696). Including patients who had crossed over from other treatment groups, the cumulative response rate of CsA, CS, combination of CS and CsA, was 73.7% (14/19), 46.7% (7/15), 83.3% (5/6), respectively (P=0.193); the cumulative rate of CR was 26.3% (5/19), 26.7% (4/15), 66.7% (4/6), respectively (P=0.202). In 23 refractory and relapsed PRCA patients, 8 out of 12 (66.7%) refractory patients and 4 out of 11 (36.4%) relapsed patients achieved remission. The response rate of treatment with traditional immunosuppressive agents (CS and/or CsA) was higher than other immunosuppressive agents (65.0% vs 20%, P=0.014).

Summary/Conclusions: CsA and/or CS are effective similarly in treating PRCA. Adult patients with relapse or refractory PRCA, there were no satisfactory treatment measures if CsA and/or CS were not be administered or un-effective. It was still needed to explore a more effective therapy for them.

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, antihepatitis 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. Treatment (ETV or lamivudine) was started when HBV reactivation (defined as detectable HBV DNA) was encountered or as a anti-viral prophylaxis regimen for some patients (anti-HBc positive). 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 found no HBV reactivation. All the 48 patients with negative HBV 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 SYNDROME PATIENTS

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Background: Shwachman-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: Aim of the study was to find out the predictive clinical and laboratory characteristics of SDS patients.

Methods: The patients who were sent to Hacettepe Inherited Bone Marrow Failure Center for molecular work-up between June 2015 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 was detected in ultrasonography of that patient. Another patient (25%) with SDS had skin problems and anemia (75%) of the patients had failure to thrive. Three patients (75%) had anemia associated to neutropenia, and 1 patient (25%) had pancitopenia 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 bicytopenia, 10% had pancitopenia. 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 bicytopenia or pancitopenia 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 anaemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH) are included, together with other pathologies, within the bone marrow failure syndromes (BMFS). In the present report, these clinical entities are understood as independent pathologies, due to the extremely frequent evolution in the clinical course of patients with PNH with pancytopenia and/or AA with a developing PNH clone. Firstly, clinical, laboratory and treatment data of the patients who were diagnosed with PNH or AA were reviewed. Afterwards, patients with PNH and AA were compared to those of control group.

Methods: A clinical form was elaborated and distributed among the investigators of the PNH Spanish Registry. Clinical, laboratory and treatment data of the patients 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 patients who suffered from PNH with pancytopenia were mostly male, there was a male/female ratio of 8/7. In the cases under study, the clinical presentation of PNH and AA differed significantly (Table 1). However, the same clinical presentation was observed in 7 patients (20.5%) of both syndromes. 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 bicytopenia or pancitopenia might be the hematological presentational findings of SDS.

Table 1. Heavy metal levels in patients and control group.

<table>
<thead>
<tr>
<th>Metal</th>
<th>PNH (n=17)</th>
<th>Control (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium (mg/L)</td>
<td>2.4 (1.5-3.5)</td>
<td>2.2 (1.4-3.6)</td>
<td>0.89</td>
</tr>
<tr>
<td>Cobalt (mg/L)</td>
<td>0.0 (0.0-0.1)</td>
<td>0.0 (0.0-0.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Copper (mg/L)</td>
<td>1.4 (0.8-1.8)</td>
<td>1.4 (0.8-1.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>2.1 (1.4-2.9)</td>
<td>1.7 (1.2-2.1)</td>
<td>0.89</td>
</tr>
<tr>
<td>Selenium (mg/L)</td>
<td>0.0 (0.0-0.1)</td>
<td>0.0 (0.0-0.1)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Clonal evolution in AA is frequently associated with the development of a PNH clone at the time of diagnosis, throughout the pathologic natural course or even after disease’s resolution. The development of such clones has been related to better prognosis in AA right after the immunosuppressive therapy (IST). Our experience demonstrated the presence of hemolysis 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 generally) were evaluated retrospectively. Autoimmune cytopenias such as ITP and AIHA were detected in 9 (5.16, 07) 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 OSAS:1 patient), both ITP and AIHA were observed. Immunosuppressive therapy with steroid, cyclosporine, mycophenolate mofetyl 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 autoimmune. The reduction of central and peripheral tolerance is held responsible for autoimmune in PID.

Summary/Conclusions: As a conclusion, we wanted to point out autoimmune cytopenias in patients with PID and the requirement of multidisciplinary approach for treatment.
Table 2. Classified heavy metal level in patients and controls.

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uranium</td>
<td>Normal</td>
<td>Low</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Normal</td>
<td>Low</td>
</tr>
<tr>
<td>Copper</td>
<td>Normal</td>
<td>Low</td>
</tr>
<tr>
<td>Zinc</td>
<td>Normal</td>
<td>Low</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 patients. Changes in the hematologic presentation and clinical outcomes of PNH patients can be diagnosed in geriatric population. Comorbidity is more prevalent in geriatric population. Geriatric patients have increased 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 comorbidity on the PNH setting. To evaluate the use of eculizumab in geriatric age patients.

Methods: In a multicentric retrospective study, Cumulative Illness Rating Scale (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 (18.8%) 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 geriatric cohort: 3-13) Age and comorbidity were poorly correlated (p=0.0187, R-square 0.15). No differences in clinical presentation. Pathological examination of the bone marrow was compatible with PRCA. Parvovirus IgM and DNA was negative; IgG was found to be positive. Because of the lack of response to steroids, Rituximab was given (375 mg/m², weekly). Anemia and patient’s clinical condition improved after 8 weeks of treatment.

Background: Lymphoproliferative disorders (LPD) constitute a heterogeneous group of diseases related to expanding polyclonal or monoclonal lymphoid cells in the setting of immune dysfunction. EBstein-Barr virus (EBV) has been implicated in the development of a wide range of B-cell LPD spectrum. EBV associated LPDs (EBV-LPD) are more commonly encountered after stem cell and organ transplantations. Pure red cell aplasia (PRCA) is an uncommon disorder characterized by a severe normocytic anemia due to erythroblastopenia in an otherwise normal bone marrow. PRCA may be primary or develop secondary to viruses, autoimmune diseases, hematological malignancies, thymoma, solid tumors and drugs. Aims: A case, who was diagnosed with EBV-LPD and developed PRCA during follow-up, is presented.

Methods: A 75-year-old woman with pain in upper and lower extremities applied to our center in February 2016. Her past medical history was unremarkable except for rheumatoid arthritis. On physical examination bilateral cervical, submandibular, axillary lymphadenopathies (LAP) and splenomegaly were detected. Laboratory tests revealed normochromic normocytic anemia, elevated serum lactate dehydrogenase and acute phase reactants. Positron emission tomography (PET) showed supra- and infradiaphragmatic malignant lymph nodes and splenic involvement. An excisional biopsy of cervical LAP was performed. Pathological examination showed CD20 (+) and CD30 (+) large B cells in the interfolllicular area. EBV early RNA signals were checked by in situ hybridization and viral transcripts were detected. Diagnosis of EBV-LPD was made. During diagnostic work-up deepening of anemia with reticuloctopenia, increased transfusion requirement and inadequate response to transfusion necessitated a bone marrow aspiration and biopsy. Pathological examination of the bone marrow was compatible with PRCA. Parvovirus IgM and DNA was negative; IgG was found to be positive. Because of the lack of response to steroids, Rituximab was given (375 mg/m², weekly). Anemia and patient’s clinical condition improved after 8 weeks of treatment. Results: In the pathogenesis of LPD polyclonal lymphoid response to an antigenic trigger is thought to be followed by development of monoclonal neoplastic diseases. In our case, this trigger was thought to be EBV as it is known as one of the main causative agents for LPD in the literature. Clinical complaints and physical examination findings are common among all patients and frequently not leading to a definitive diagnosis in most of them as it is the case in our patient. Compared to the strong association of secondary PRCA with parvovirus B19 its association with EBV is rare. PRCA can develop before the diagnosis, during the course and after the remission of LPD. In our case we observed PRCA in the follow-up period of EBV-LPD. Summary/Conclusions: On the basis of EBV-LPD being more common in transplant setting our case was thought to be unique due to the absence of transplantation or immunosuppression history. This case report points out to the possibility of occurrence of two rare diseases, EBV-LPD and PRCA.
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 unmutedIGH as compared to patients with negative FISH. They also showed shorter progression free survival (PFS), treatment free survival (TFS) and overall survival (OS).

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 concerning events deaths due to the haematological disease.

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 unmutedIGH remained significantly associated 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 1.12-2.17; p=0.003) and TFS (HR 1.22; 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 148 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 CONFRONS 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-)) on 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 (442/650). The most frequent abnormality was 13q-, observed in 302 patients (47%), but as a sole alteration in 212 cases, followed by +10 (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 Döhner 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 1.

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.

haematologica | 2017; 102(s2) | 707
Background: Chronic Lymphocytic Leukemia (CLL) pathogenic 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 MethylScreen™ in the CFX96Biorad Real-Time PCR system. For this purpose, we used EpTect Methyl II PCR Assay which enables us to calculate the methylated and unmethylated fraction after simultaneous digestions with specific restriction endonucleases. Methylation analysis of RAD21 gene promoter was performed on unmethylated and methylated CpG islands using RT-PCR. FISH analysis was carried out using the commercial FISH sets for detection of the most common abnormalities of the disease including deletions of 13q14 (TP53), 11q22.3 (ATM) and 13q14.33/13q34 (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 abnormalities detected by karyotyping and/or FISH analysis. Methylation study was successful in all healthy donors and in 101 out of 105 CLL patients. All healthy donors had normal RAD21 gene promoter methylation, whereas 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=4.25, 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) showed high methylation rate score, >90% of the analyzed cells. Stratification of patients according to cytogenetic 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 13 (8/24), in 33.33% (4/12) with del(13q), in 31.25% (5/16) with -17del(17p), 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 chromosomal 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. Identification of the epigenetic landscape of CLL may help in the design of new targeted therapeutic agents.

PB1763

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 the most prevalent leukemia in the western adult population. Although advanced age, white ancestry, and family history of hematologic malignancies are risk factors, the etiology of CLL still unknown. One of the mechanisms associated with the development of this pathology is related to the oxidative stress (OS) resulting from an imbalance between the production of reactive oxygen species (ROS) and their disposal by the antioxidant defenses. The number factor erythroid 2-like gene type-2 (NFE2L2) and its suppressor, the Kelch-like ECH-associated protein 1 (KEAP1) gene, plays a central role in ROS balance. Changes in these genes, whether due to somatic mutations or genetic variants (SNPs), have been associated with some hematological diseases. However, the role of NFE2L2 and KEAP1 genes polymorphisms in susceptibility and prognosis is still not studied.

Aims: To assess the role of two SNPs in the NFE2L2 and KEAP1 genes on CLL susceptibility, their influence on prognosis/survival, and their correlation with clinical and laboratory characteristics of patients.

Methods: Genetic variants rs13001694 (NFE2L2) and rs11085735 (KEAP1) were genotyped by tetra-primers-AMRS-PCR in 176 patients with CLL and 261 controls. The role of these gene polymorphisms in CLL susceptibility and their association with clinical and laboratory characteristics as well as with therapy response was assessed by logistic regression analysis and/or by Fisher’s exact test. The influence on prognosis and survival was performed through Kaplan-Meier analysis and log-rank test using Cox regression curves by estimating the progression free survival (PFS) and the overall survival (OS).

Results: The results showed that individuals with the GG genotype (NFE2L2) are at higher risk of developing CLL (Odds ratio (OR): 2.03; 95% confidence interval (CI): 1.234-3.51; P=0.004). In addition, 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 1.063-2.393, p=0.037; KEAP1 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, 1.119-3.887, p=0.026).

Finally, the overall survival of CLL patients appears to be influenced by the genotypic profile of NFE2L2 / KEAP1 [GP AG / AC patients have a lower mean survival (29.2±13.6 months) compared to other GPs (40.7±12.4 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=0.022)].

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 (5.4±3.5%). By MN analysis, an increased frequency in CLL patients (2.81±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 2.8±1.3%, 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 MONOCONAL ANTIBODIES DISPLAY SPECIFIC ALTERATIONS IN GENE EXPRESSION PROFILE
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Background: CD20 monoclonal antibodies (mAb) 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 immunoprecipitation 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 maximal dose of mAb. We could identify in these cells CD20 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 with the increase of CD55 and CD59.

Summary/Conclusions: In summary, CD20 triggering by therapeutic mAbs initiates a signaling cascade that results 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.

This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (L16010) and by the research grant AZV-MZ CR 15-33561A-4/2015 and grant MUNI/A-1106/2016.

PB1766
DIFFERENTIAL EXPRESSION PATTERNS OF CHEMOKINE RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA
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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, CXCR5, CXCR7, 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-CR5, CCR8, CCR9, CXCR1, CXCR2, CXCR7) 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, CXCR7 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 CXCR7 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 comparing 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.


PB1767
RESIDUAL SERUM CONCENTRATIONS OF RITUXIMAB ARE ASSOCIATED WITH RELAPSE RISK IN CHRONIC LYMPHOCYTIC LEUKEMIA
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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 immunomunotherapy, which is the aim of the present study.

Methods: Residual serum concentrations of rituximab were determined by an enzyme-linked immunosorbert 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), with 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 administered cycles. Early relapse was associated with lower residual rituximab concentration (AUC) (AUCmeanA=1.28±1.01 mg/l*day, AUCmeanB=2.79±1.93 mg/l*day, p=0.02). Additionally, the residual rituximab 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 higher expression of CD38 and a more frequent administration of 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.

Summary/Conclusions: In conclusion, serum residual rituximab 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, IDELAISILIB 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 (ADCP) 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 antiproflliferative or cytotoxic effects of the TKIs, idelalisib 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 ME-1 target cells but had strong antiproliferative effects. In contrast, venetoclax induced strong cytotoxicity on ME-1 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 addition of ibrutinib, 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 an accumulation of morphologically mature monoclonal lymphocytes B with the 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 associated with higher Rai stage, increased concentration of lactate dehydrogenase and beta-2 microglobulin, and more rapid progression of the disease. The number of lymphocytes B CD19+ZAP-70+ correlated positively with the number of CD19+PD-1+ B cells, CD4+PD-1+ T cells, and CD8+CD200+ T cells.

Summary/Conclusions: The study confirmed the association between unfavorable prognosis and high expression of exhaustion markers in CLL patients. Determination of PD-1+, PD-L1+, CD200+ and CD200R+ lymphocytes T and B constitutes valuable diagnostic tool, completing cytometric evaluation of CLL.

PB1770

HSP70 AND HSFG1 GO HAND IN HAND AND HAVE A ROLE IN THE SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA NEOPLASTIC 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 pro-survival role of HSP70 in cancer, we were aimed at characterizing this protein and its master regulator, the Heat Shock Factor 1 (HSF1), within the pathogenetic mechanisms leading to CLL.

Methods: HSP70 and HSF1 expression levels were evaluated by Western blotting (WB) analysis in leukemic and normal B cells. HSP70 and HSF1 protein levels were correlated with the number of CD19+PD-1+ B cells, CD4+PD-1+ T cells, and CD8+CD200+ T cells.

Results: We demonstrated that HSP70 and HSF1 are overexpressed in leukemic vs normal B cells and their expression levels correlate to poor prognosis in CLL. We also analyzed HSP70 and HSF1 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 HSF1 are localized in the nucleus of CLL B cells. HSP70 and HSF1 inhibition was proved to be effective in inducing a dose-dependent in vitro apoptosis of CLL cells.

Summary/Conclusions: HSP70 and HSF1 overexpression and correlation with poor prognosis in CLL patients underlines their pivotal role in the regulation of leukemic B cell survival. HSP70 and HSF1 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 HSF1 are both localized into the nucleus after stress conditions, however we found both HSP70 and HSF1 localized into the nucleus of CLL B cells. 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, HSF1. We observed that both inhibitors, Zafirlukast and Fisetin, lead to an in vitro dose dependent cell apoptosis. These data demonstrate HSP70 and HSF1 involvement in the pathogenesis of CLL and identify HSP70/HSF1 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 predicts 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 RQ-PCR methodology, using TaqMan chemistry and Abl as an endogenous control gene. Quantification of target gene expression was made by comparative ΔΔCT method using hL-80 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, 0.033±0.025 and 0.157±0.257, respectively. The level of SLC28A3 expression was not associated with the 17p deletion 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 LEUKEMIA 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 unmutated 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 (53 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: c.711C>A (p.R238H) and c.1712A>G (p.N571D) mutations. TP53, NOTCH1 and SF3B1 gene, in exons 4, 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 found in IR-exposed patients in comparison with the control group (6.7% vs 17.7%; p=0.012). Other features were found among IR-exposed CLL patients also. Specifically, TP53 mutations were seen with equal frequency among mutated (11.1%) and unmutated (11.8%) IGHV cases in IR-exposed CLL patients, while the tendency to prevalence of TP53 mutations in unmutated 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 SF381 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.

PB1773

DRUG SENSITIVITY SCREENING IN CHRONIC LYMPHATIC LEUKEMIA AND MULTIPLE MYELOMA FOR PERSONALIZED CANCER THERAPY

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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 with cancer is that origin of each cancer is a clan event evolving into tumor heterogeneity. We focus on Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma and Follicular lymphoma (FL) that are currently considered incurable, through current treatment regimens that cannot eradicate ALL myeloma patients, CLL and MM cancer eventually relapse. Current challenges in using therapies 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 (n=1).

Aims: To introduce individualized treatment for patients against available therapies, we aim to established cell-based assays and drug sensitivity platform at NCMM, 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 CAR, APRIL and BAFF for 24 hours stimulation. We perform drug sensitivity screening with Premistimulated CLL cells in 384 well formats without feeder cells. We culture MM cells in 384 well format for drug screening in response to Thelp cells pretimulation in the presence of IL2. To support high-throughput drug sensitivity screening. We use cell-based assays such as CellTiter-Glo® Cell Viability Assay and CellTox™ Green Cytotoxicity Assay to define drugs that inhibit cancer cell growth. Additional methods such as cell proliferation assay, CellTox Green, apoptosis and oxidative stress (glutathione release) are also applied. We also used established cell barcoding on CLL/MM for flow cytometry (7-AAD/BrDU cell proliferation and Caspase8/9 apoptosis assay).

Results: Standard Curve for cell proliferation, 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-pretreated) has been performed for 48, 72 hrs and 5 days) using cell proliferation, CellTox-Green assay for M2 cells has been performed. Benzalkonium chloride (BzCl) 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).

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 flow cytometry 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. Chemomunotherapy 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 adenine for thymine at position 131(0.6%). The FCGR2A-131H allele has a higher affinity for Fc gamma receptor than 131R variant. The gene for FCGR3A has also two polymorphic variants: 158 valine (V158) and phenylalanine (F158) due to a single base substitution of nucleotide thymine for adenine at position 158 (0.6%). The 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 cytotoxicity (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 62.7(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 68.5(34-173)x10⁹/L. Percentages 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: Distribution of genotypes in our patients showed: 32% H/H, 49% H/R and 19% 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=0.001) or FCGR3A (p=0.109) in CLL patients with complete, partial or no response to R-FC treatment (Table 1).

Table 1. Genotype distributions for FCGR2A & FCGR3A in patients with CLL.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>No Response</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>FCGR2A</td>
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</tr>
<tr>
<td>131H/H</td>
<td>20.6% (22.7%)</td>
<td>63.7% (56.6%)</td>
<td>15.7% (11.1%)</td>
<td>0.609</td>
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<tr>
<td>FCGR3A</td>
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<tr>
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Summary/Conclusions: Our results are similar with previously published 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 patient’s population.

PB1774

FCGR2A AND FCGR3A VARIANTS ARE NOT ASSOCIATED WITH RESPONSE TO RITUXIMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Aims: In this study, we analyzed the mutation status and pattern of IGHV,IGHD and IGHJ gene usage in Macedonian CLL patients.

Methods: Ninety-seven consecutive CLL patients that presented at the University Clinic of Hematology –Skopje in the period between 2011-2013, were included in the study. IGHV mutation status and gene repertoire were analyzed using the reverse transcriptase– polymerase chain reaction (RT-PCR) and sequencing methodology. The mutational status of the IGVH genes was determined using two databases: IMGT/TV-QUEST tool and IgBLAST software. The stereotyped subset assignment was performed using ARRestiAssignSubset tool (Bioinformatics Analysis Team).

Results: We found that 44.3% of the cases belonged to M-CLL and 55.7% to U-CLL, with a progressive disease dominant in the U-CLL subset. Both groups were comparable regarding the age and gender distribution. Only 39% of the M-CLL patients presented with a progressive disease, compared to 74% of the U-CLL patients (p<0.05).The comparison of median time to the first treatment (TTT) between M-CLL and U-CLL (39 months versus 8 months, respectively) showed a statistically significant difference between the groups (p<0.01). The most frequently expressed IGHV genes were: IGHV1-27 (28.9%), IGHV4-37 (23.7%), IGHV5 (2.0%), and IGHV2-23 (1.0%). Among 32 different IGHV genes, 8 genes were found (V1-46,V1-69,V3-21,V3- 23,V3-30,V3-33,V3-48 & V4-34) in 58.8% of all cases, revealing a strong bias in IGVH gene expression in CLL. IGHV1-69 was the most frequently expressed gene of all (16.5%), and exclusively found in the U-CLL group demonstrating a frequency of 29.6%. The IGHV3-21 was detected with a low frequency of 4.1%, as reported for CLL patients from other Mediterranean countries. The distribution of IGHD subgroups was as follows: IGHD3, 52.6%; IGHD2, 17.5%; IGHD6, 13.4%; IGHD1 7.2%; IGHD4 7.2%; and IGHD5 2.09%. The most frequent IGHJ gene was IGHJ3 (49.4%), followed by IGHJ5 (23.7%), IGHJ6 (11.4%) and IGHJ2 (3.1%), IGHJ1 (2.0%). In 10% of the cases, the VHCD3R amino acid sequences belong to previously defined stereotyped clusters. Only one of the rearrangements with stereotyped VH-D3R belonged to the M-CLL subset.

Summary/Conclusions: Our study showed a strong correlation between IGHV gene mutational status and clinical course of CLL. Results on IGHV-IGHD-IGHJ genes usage in our study are comparable to the previously reported from Mediterranean countries. The high frequency of V1-69gene and low frequency of IGHV3-21 in our CLL patients that originate from a small geographic region further promotes the geographic bias in the use of IGVH genes and points to an important role in antigen stimulation in the pathogenesis of the CLL subsets. Our findings indicated a lower expression of the stereotyped BCR region than those previously reported (~30%), but they were comparable with the results reported for the Serbian CLL patients (10.1% versus 15.3%, respectively), in the only previous published study of this kind from Western Balkans.
Chronic lymphocytic leukemia and related disorders - Clinical

PB1776

LAMBDA LIGHT CHAIN RESTRICTION – USEFUL FOR HAIRY CELL LEUKEMIA PROGNOSTICATION?

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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: Prognostic factors are 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 the 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 was 3 years. The OR rate for 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 no statistically significant, was observed in patients achieving CR (namely MRD negative) and without thrombocytopenia at presentation. Excitingly, the 61% of patients with CLL and Evans-Fisher syndrome, along with 15 CLL patients without 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: HCL remains an effective treatment of patients with CLL associated with severe thrombocytopenia 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 perisplenic adhesions, amidst portal hypertension and thrombocytopenia are considered to be special surgical risk factors in this patients.

PB1777

MONOCOCLAL B-CELL LYMPHOCYTOSIS IN THAI POPULATION: PREVALENCE AND IMMUNOPHENOTYPIC CHARACTERISTICS

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Background: Monoclonal B-cell lymphocytosis (MBL) is characterized by the presence of <5x10^9 clonal B-cells/L in peripheral blood (PB) in otherwise healthy subjects, in the absence of symptoms and signs of a B-cell lymphoproliferative disorder (LPD). MBL is considered a precursor to chronic lymphocytic leukemia (CLL) and other B-cell malignancies.

Aims: To study the immunophenotypic features and prevalence of MBL in healthy Thai individuals.

Methods: Peripheral blood (PB) samples from 616 healthy Thai individuals (313 female), 18-80 year-old with normal lymphocyte counts were immunophenotyped using high-sensitive flow cytometry, based on 5-color screening for >5 x 10^6 total PB leucocytes. The initial PB samples were screened for clonal B cells using MultiMix Triple-Color Reagent (Kappa Light Chains/FITC, Lambda Light Chains/RPE and CD19/RPE-Cy5). In those cases in which a clonal B cell population was detected by imbalanced of sIgK:sIgL expression.

Results: Of total 616 subjects, MBL was found in 8 cases (1.2%) including 3 and 5 male and female cases respectively. Among 40 years or older, MBL was found in 5 out of 448 cases (1.1%). Compared with non-MBL group, subjects with MBL were significantly older (101.3 years versus 71.7; p=0.049) and had a significant higher number of absolute and B-lymphocyte count (median 3.1 versus 1.6 X 10^9/L; p=0.03 and 0.35 versus 0.16 X 10^9/L; p=0.02, respectively) while the median white blood cell count was not different between 2 groups. Also, there were more subjects in MBL group who had history of lymphoproliferative diseases (LPD) 37% vs 0% (p=0.01) and influenza vaccination within 2 years (50% vs 8.7%; p=0.003). Among 8 cases with MBL clone, 6 cases had low-count MBL (<0.5x10^9 clonal B-cells/L) while only 2 cases had high-count MBL (>0.5x10^9 clonal B-cells/L). All 8 cases had persistent positivity of MBL clone after treatment was repeated within 3 months after the initial test. In follow up test, only 1 case with initial high-count MBL had decrease number of B cell clone and became low-count MBL. There was not significant different in age between subjects in low and high-count MBL group. Six cases had typical CLL phenotype MBL clone (CD5+, CD23+, CD20+dim and light chain restriction). Whereas 1 case had atypical CLL phenotype MBL (CD5-, CD20+dim and light chain restriction). 1 case had atypical MBL phenotype MBL (CD5-, CD20+dim and light chain restriction).
PB1779

SPONTANEOUS CLINICAL REGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 9 CASES FROM THE ERIC REGISTRY

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Background: 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); 13 pts had unmutated IgVH and 2 had 17p deletion. The median observation period was 9 months. No patient received concurrent antimalplatelet or anticoagulation therapy.

Methods: Nineteen pts achieved a partial response and an increase of hemoglobin and lymphocyte count in 5/9 cases 5 months after crizotinib start. In 5/9 cases 5 months after crizotinib start. In 5/9 cases 5 months after crizotinib start.

Results: Nineteen pts achieved a partial response and an increase of hemoglobin and lymphocyte count in 5/9 cases 5 months after crizotinib start. In 5/9 cases 5 months after crizotinib start. In 5/9 cases 5 months after crizotinib start. In 5/9 cases 5 months after crizotinib start.

Summary/Conclusions: Our study showed minor bleedings in pts treated with IBR. A severe impairment of collagen-induced aggregation was caused by IBR and was countering the mild phenotype in treated pts, which 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.
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. The purpose of this study was to evaluate the outcome in a group of patients given cladribine, 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.

Aims: In this retrospective study, we 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=0.4). Median days of hospitalization were 8 for both groups (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 study is the first comprehensive summary of the natural history 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 heterogeneous PR (HPR) group (Hallek 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 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 the potential evolution of clinical stage, and enable discrimination of 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 thrombocytopenia 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 scintigraphies of CLL patients were performed. Demographic data, Rai-stages, and established thyroid disorders were recorded.

Results: One hundred CLL patients were included into the study (65 male, mean age was 62.9±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 low 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 according 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 sickle 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, and was positive in 6 patients of ≥65 years old age group (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.
and was positive in 11 patients in ≥65 years old age group (p=0.053). There was a statistically significant difference in thyroid function tests according to the Rai-stages, ages and sexes.

Summary/Conclusions: We determined that incidence of hypothyroïdism or hyperthyroidism associated with all reasons do not increase in patients with CLL when compared with general population. However, we also determined that the incidence of Hashimoto thyroiditis was higher than general population (incidence of Hashimoto thyroiditis in general population is 2-5%). Anti-TG positivity was also higher than general population (positivity of anti-TG in general population is 5-20%). In addition, the positivity of 2 antibodies increased with advanced ages. Patients with CLL—especially the elderly cases—both sexes and all Rai-stages should be examined for thyroid gland disorders, mainly for Hashimoto thyroiditis.

PB1784

CLINICAL-BIOLOGICAL CHARACTERISTICS, TREATMENT OUTCOME AND SURVIVAL OF SMALL LYMPHOCYTIC LYMPHOMA PATIENTS: A REAL-LIFE EXPERIENCE

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Background: Studies of B-SLL published to date have included heterogeneous groups of patients(pts) and did not use modern diagnostic criteria, or included pts who had in fact chronic lymphocytic leukaemia. Outside the context of clinical trials, SLL pts are treated heterogeneously and thus there are no data concerning the impact of different treatment approaches on response and survival. In the updated WHO classification it is pointed out that there are a subset of 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 (size of LN and presence of PCs) with the disease itself, may impact quality of life (QoL). Patient Reported Outcomes (PROs) in daily clinical practice is a resource-intensive procedure and may be affected by low adherence, risk of recall bias and difficulties in establishing reproducible procedures. 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.

Methods: Heminsight was implemented at our Centre to collect PROs from CLL patients in daily practice.

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 attraction: the study was proposed to 91 consecutive patients, 16, SF-36, and the eight-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 received regular reminders by email to complete the tasks. The following measurements will be assessed: system attraction (percentage of CLL patients adhering to the project); patient compliance in filling out questionnaires; system efficiency (number of alerts related to QoL worsening and number of questionnaires not submitted) and system effectiveness (significant differences in changes in QoL scores from diagnosis to response/relapse, changes of therapeutic approach/action following an alert, changes in adherence of therapy).

Summary: 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 attraction: the study was proposed to 91 consecutive patients, 16, SF-36, and the eight-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 received regular reminders by email to complete the tasks. The following measurements will be assessed: system attraction (percentage of CLL patients adhering to the project); patient compliance in filling out questionnaires; system efficiency (number of alerts related to QoL worsening and number of questionnaires not submitted) and system effectiveness (significant differences in changes in QoL scores from diagnosis to response/relapse, changes of therapeutic approach/action following an alert, changes in adherence of therapy).

Table 1.

<table>
<thead>
<tr>
<th>PROs administered to patients with CLL</th>
<th>Response/adherence</th>
<th>Treatment</th>
<th>Total %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total %</td>
<td>24/36</td>
<td>16/24</td>
<td>72%</td>
<td></td>
</tr>
</tbody>
</table>

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 and is seen in all 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 in a real-world setting.

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, outpatients and CLL-drug costs, incurred while treated with the index treatment were 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 3.51 in the bendamustine cohort (p=0.581). During treatment, 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 new 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 long-term preservation and the deepening of the therapeutic response, if it is possible. This problem can be solved by intensification of therapy (including autologous transplantation of hematopoietic stem cells) or maintenance therapy (MT).

Aims: To estimate the importance of maintenance therapy in the treatment of patients with CLL.

Methods: The study included 198 patients. Male to female ratio - 1.3:1. We have used NCI revised guidelines (Hallek M, 2008) for treatment initiation, assessment of residual disease and minimal residual disease (MRD). Induction chemotherapy was conducted under the following programs: RB, FC, RFC, R-CHOP, Ibrutinib-RB, Ibrutinib-R. Evaluation of MRD was performed using 5-color flow cytometry of the bone marrow samples. The maintenance therapy was conducted 144 (72.7%) patients. Rituximab 500 mg/m² intravenously every 8 weeks (n=116) for 2 years; Ibrutinib 420 mg, orally, daily (n=28) continuously. The remaining patients (n=54) were under dynamic observation without therapy.

Results: The increasing of depth of response (from partial (PR) to complete remission (CR)) was observed only in group of patients receiving MT – 10.4% (15/144) (p=0.013). The frequency of increase the depth of remission in the patients treated with MT of Ibrutinib was 28.6% (8/28), MT of Rituximab – 6.0% (7/114) (p=0.0005). The medians of PFS and duration of response were a longer in the patients with MT versus in the patients without MT: PFS – 48 months and 37 months, respectively (p=0.03); duration of response – 44.0 months and 25.5 months, respectively (p=0.006). The median of duration of response in the patients with MT of Ibrutinib was not reached, in the patients with MT of Rituximab – 41.9 month, in the patient without MT – 25.5 month (p=0.004). The frequency of relapses in the group of patients with MT was 39.6% (57/144), in the group of patients without MT – 66.7% (36/54) (p=0.0007).

Recurrence of the disease occurred more frequently in the group of patients treated with BR and bendamustine, compared with Ibrutinib: 45.7% (53/116) and 14.3% (4/28), respectively (p=0.002). The median duration of observation in the group with rituximab was 22 months, while in the group with Ibrutinib – 11 months. MRD was not detected after 6-12 months of MT in 23.5% (12/51) had previously MRD-positive patients. Among patients with MRD-negative CR relapse is less common than in patients with BR and bendamustine – 20.0% (4/20) versus 62.5% (10/16), respectively (p=0.009). Significant differences in the incidence of infectious complications between patients with MT and without were not detected (p=0.05) (Figure 1).

Figure 1.

Summary/Conclusions: The conducting of MT patients with CLL allows to achieve increasing the depth achieved remission and increase the duration of its preservation. MT may be a means of control over the minimal residual disease and the method of its eradication.

PB1788
MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEOFLOW LYMPHOCYTE SCREENING TUBE (LST)

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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 up 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 evaluative specimens of the original LST clinical trial were regressed 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 FACSDuo II flow cytometer with the 4-2H-2V CE-IVD configuration and LST template v2.0.

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
were collected in EDTA or heparin anticoagulants or PBS (for lymph nodes) at three external study sites. Informed consent was not required in the clinical study. All specimen in the original study were simultaneously stained with investigational BD OneFlow LST and comparator EF liquid reagent system was concordant in delineating patients into normal (no follow-up) or follow-up, and 100% overall agreement in identifying B-, T-, NK, and other cell-lineage with 86.1% CI. Flow cytometry analyses were performed on a BD FACSCanto II instrument using LST v2.0 template. The multisite performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was concordant in identifying abnormal from normal normale populations in patients with CLPDs. BD OneFlow LST is for in Vitro Diagnostic Use. CE Marked to the European in Vitro Diagnostic Medical Device Directive 99/7/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 all three classes of immunoglobulins (IgG, A and M) are involved. Recently, a novel assay for detecting heavy/light chain (hevy/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, Isotypes of heavy/light chain: IgGk kappa, IgG lambda, IgAk kappa, IgA lambda, IgM kappa, IgM lambda and Free light chain: kappa (K), lambda (L), ratio of K/L and calculation of ratios of monoclonal/polyclonal immunoglobulin (HLC ratio).

**Results:** The total cohort constituted of 126 "treatment - naïve", patients with CLL and 26 healthy volunteers. Median age was 64 years, 64% were males and 78% and Binet stage A, while 19% and 3% were stages B or C respectively. Significant different in HLC immunoglobulin subunits (IgG, A and M) were identified in CLL patients compared to healthy controls (p value of 0.001, 0.005 and 0.001 respectively). Abnormal IgG-lambda values were evident in 15 patients (10%) and associated with more pronounced leucocytosis (p=0.005), higher B2mg levels (p=0.022) and the presence of 17p delletion (p=0.05). Median B2mg lambda was also significantly in 38% and 56% of cases respectively compared to 8% and 9% in the controls. For IgG subclasses: both IgG2 and IgG4 levels were significantly higher in CLL patients than in healthy controls (p=0.01 and 0.001 respectively). In addition , IgG2 and IgG4 were also confirmed to be significantly lower in CLL patients than in controls (p=0.001 and 0.002 respectively). Median B2mg levels were detected in 15 patients (10%) and associated with more pronounced leucocytosis (p=0.022) and the presence of 17p delletion (p=0.05). Median B2mg lambda was also significantly in 38% and 56% of cases respectively compared to 8% and 9% in the controls. For IgG subclasses: both IgG2 and IgG4 levels were significantly lower in CLL patients than in healthy controls.

**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 normale populations in patients with CLPDs. BD OneFlow LST is for in Vitro Diagnostic Use. CE Marked to the European in Vitro Diagnostic Medical Device Directive 99/7/EC. 23-19566-00.

**PB1790**

**INFLUENCE OF TREATMENT ON CONCENTRATION OF CYTOKINES IN BLOOD OF PATIENTS WITH HAIRY CELL LEUKEMIA**

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**Background:** A pathogenic role and proinflammatory role of cytokines in treatment of patients (pts) with hairy cell leukaemia (HCL) are not fully established.

**Aims:** to define the concentration of cytokines such as TNFα, IL-6, sIL-2R, TGFβ1 in serum of HCL pts before and after treatment with IFNα or 2-CdA and to estimate the relationship with blood count indexes in HCL pts.

**Methods:** The study group consisted of 26 primary pts with the classic variant of HCL (median age - 47 years). A control group consisted of 12 healthy persons (median age - 50 years). The concentration of cytokines was measured using a validated commercial ELISA kits.

**Results:** Median of TNFα content in serum of HCL pts before treatment was substantially lower (3.57 pg/ml) than in healthy persons (8.36 pg/ml; p=0.275), however levels of IFNα and 2-CdA did not influence TNFα level. Median of TGFβ1 concentration in serum of HCL pts was also significantly lower, than in healthy persons (265.52 pg/ml and 156.22 pg/ml respectively; p=0.0004). Reliable increase of TGFβ1 concentration was observed only after 2-CdA therapy (928.33 pg/ml; p=0.281). Cross-correlation relationship was revealed between the TGFβ1 concentration and the level of haemoglobin (r=0.23; p=0.1) as well as with leucocyte count in HCL pts (r=0.24; p=0.09). Median of IL-6 content in serum of HCL pts before treatment was higher, than in healthy persons. Theraphy with IFNα or 2-CdA reduced IL-6 level to the control values. Certain cross-correlation relationships were revealed between the IL-6 level and percentage of hairy cells in bone marrow (r=0.33; p=0.01). Median amount of lymphocytes in peripheral blood of HCL pts (r=0.24; p=0.09). Median serum concentration of s-IL-2R (24.73 ng/ml) in HCL pts more than 20-fold exceeded such in control group (1.15 ng/ml; p=0.0000005). Cross-correlation relationship was revealed between the percentage of hairy cells in bone marrow and s-IL-2R level in serum (r=0.27; p=0.08). Obtained results may be an evidence of predominant secretion of s-IL-2R by tumor cells in HCL pts.

**Summary/Conclusions:** New data regarding pathogenetic relationship between production of certain cytokines and features of hematopoiesis in HCL pts was obtained. Between the blood level of some cytokines in HCL pts and efficiency of treatment a relationship was revealed, which is possible to use for prediction of clinical course of this disease. Moreover s-IL-2R level in blood possibly can serve as a marker of tumour activity in classic type of HCL.

**PB1791**

**PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA – CLINICAL BENEFITS OF ACHIEVING A DEEP RESPONSE TO FIRST-LINE THERAPY**

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**Background:** In recent years, there have been advances in the treatment of CLL with the approval of several novel oral agents that show improvement in PFS and OS. Additionally, some agents induce a deep response indicated by complete remission (CR) and/or minimal residual disease negativity (MRD). However, there has been limited information on the longer-term clinical benefits of achieving a deep response in a real-world setting.

**Aims:** This study aimed to characterize PFS and OS for patients who achieved a deep response to first-line therapy for CLL.

**Methods:** Patient-level data were collected between July and August 2016 from 93 oncologists/hematologists in the United States. Oncologists/hematologists who participated included patients with partial remission (PR), stable disease (SD) and progressive disease (PD), iWCLL 2008 criteria were provided to guide physicians’ assessment of treatment response. The target sample size for each response type was a priori determined based on...
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 (adjusted 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- (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-.

<table>
<thead>
<tr>
<th>Event, N (%)</th>
<th>CR (N=179)</th>
<th>Non-CR (N=151)</th>
<th>PFS (%)</th>
<th>OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression/Death</td>
<td>43 (24%)</td>
<td>75 (50%)</td>
<td>26 (14%)</td>
<td>67 (44%)</td>
</tr>
<tr>
<td>Death</td>
<td>35 (19%)</td>
<td>44 (29%)</td>
<td>14 (8%)</td>
<td>33 (22%)</td>
</tr>
</tbody>
</table>

**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 patients 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.

PB1792

**ANTI-CD ANTIBODY MICROARRAY FOR MORPHOLOGY EXAMINATION OF CIRCULATING LEUKEMIA AND LYMPHOMA CELLS**

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**Background:** Matching the morphology with immunophenotype for individual leukocytes is a major issue in diagnoses of leukemia and lymphoma due to the absence of a method for simultaneous cluster of differentiation surface antigen detection and full leukocyte morphology analysis. This problem can be solved by using a leukocyte-binding antibody microarray.

**Aims:** We describe an anti-CD antibody microarray on a transparent support for leukocyte sorting and a method for preparation of the microarray-bound cells for high-resolution morphology analysis. The aim of the work was to demonstrate, that the leukocyte binding is highly specific and that the microarray-bound peripheral blood mononuclear cells both from healthy donors and patients with B-cell leukemia and lymphomas are morphologically identical to the same cells in blood smears.

**Methods:** Anti-CD antibodies were immobilised on plastic coverslips in spots 2 mm in diameter. In order to study the peripheral blood mononuclear cells (PBMC) and the mononuclear fraction separated by density gradient from peripheral blood are incubated with the microarray in non-mixing conditions at 4°C. After the unbound cells are washed away the microarray-bound cells are dried in a cytocentrifuge and stained after May-Grünwald-Giemsa for morphology examination. Using this technique we have studied the PBMC from 55 healthy donors and agree well with the reported immunophenotypes of corresponding neoplastic cells for high-resolution morphology analysis. The aim of the work was to develop a method for preparation of PBMC for the patients with leukemias and lymphomas agree with flow cytometry results for the same patients including doublet, apoptosis, CD11a, CD11c and CD103 in CLL, CD2 and CD11c in AML. The amount of healthy cells determined morphologically on the microarray varied from 20 to 97% of all anti-CD19-captured cells and 2 to 80% of all lymphocytes and was in good agreement with the percentages of cells with CD19/CD103 and CD19/CD11c coexpression determined by flow cytometry in the peripheral blood of the same patients by conventional constructs.

**Summary/Conclusions:** The microarray works as a "sorted smear" with cells positive for certain surface CD antigens localised in a predetermined area and permitting to apply any standard smear-oriented technique to the microarray-captured cells. Combined analysis of the pathologic cells' immunophenotype, cytochemistry and microarray morphology permits to apply any standard smear-oriented technique to the microarray-captured cells. Combined analysis of the pathologic cells' immunophenotype, cytochemistry and microarray morphology permits to apply any standard smear-oriented technique to the microarray-captured cells. Combined analysis of the pathologic cells' immunophenotype, cytochemistry and microarray morphology permits to apply any standard smear-oriented technique to the microarray-captured cells.
MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS T1 (B-CLPD T1) PANEL

B. Key1*, 2, A. Coskun2, 2, L. Cevreska1, 2, S. Trajkova1, 2, A. Dimovski2, M. Ivanovski1, 2, S. Tatic1, 2, N. Zavrcil2, 2, K. Judge2, 2, S. Kozlac1, 2, M. Popova-Labacevska1, 2, D. Dukovski1, 2, B. Kocoski1, 2, I. Panovska-Stavridis1, 2

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 Tube) 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.

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 24 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 reagent solutions. Acquisition and analysis were performed on a BD FACSCanto II instrument using BD OneFlow LST and B-CLPD T1 template 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 (positive negativity) of the abnormal 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 (p=0.014 (0.95-1.01)) 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 the relative fluorescence intensity of CD45+CD19+ aberrant populations for CD20+, CD200+, and CD23+ subsets and 99.1% agreement for the CD79b+ subset.

Summary/Conclusions: The multisite performance evaluation between the BD OneFlow system (LST and B-CLPD T1) and the comparator EF system, performed in distinct clinical scenarios and in patients with CLL from patients with other B-CLPDs, including presumptive cases of atypical CLL. The BD OneFlow B-CLPD T1 is fully standardized and validated system for aiding in the diagnosis of CLL from other B-CLPDs in PB and BM specimens. The multisite performance evaluation between the BD OneFlow LST and BD OneFlow B-CLPD T1 are CE Marked according to the European In Vitro Diagnostic Medical Device Directive 98/79/EC. 23-19567-00

RESULTS:
Our results based on molecular analysis from 100 subjects living in the same geographical area, show the presence of three major groups of clones with distinct but partially overlapping configurations of IGHV gene usage, IGHV mutational status and cytogenetic alterations. These included a group which mainly consisted of clinical advanced stage CLL with a skewed but different IGHV-associated gene repertoire (VH9-69 associated with HD3 gene and VH6 gene) and a second group which was frequently associated with complex karyotypes and poor-prognosis cytogenetic alterations, a third group enhanced in clones expressing specific IGHV subgroups (VH3-23 associated with HD2 genes and JH6 gene) with no or isolated good-prognosis cytogenetic alterations and a third group of clones with intermediate features, with prevalence of mutated IGHV genes, and higher numbers of del(13q) clones.

SUMMARY/CONCLUSIONS: These findings suggest that the specific IGHV repertoire and IGHV mutational status of CLL B-cell clones may adjust the type of cytogenetic alterations acquired and their clinical significances. Further long-term follow-up studies investigating the IGHV gene repertoire of CLL clones in distinct geographic areas and microenvironmental forces are required to validate our findings and discard or confirm the potential role of some antigen-binding BCR specificities contributing to clonal evolution.

PB1796
PROGNOSTIC SIGNIFICANCE OF SERUM BAFF, APRIL, TACI AND BCMA LEVELS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the accumulation of CD5+ B cells in the peripheral blood and bone marrow. Prognosis of B-CLL is highly variable which depends on certain prognostic parameters. Novel prognostic markers and risk assessment models are fundamental to identify high risk patients who may need early treatment. The two tumour necrosis factor family members (BAFF (TNFSF13B) and APRIL (TNFSF13B)) and their receptors [BAFF-R (TNFRSF13C), TACI (TNFRSF13B), BCMA (TNFRSF17)] play a critical role in the survival of normal B cells.

Aims: In this study, we aimed to investigate the impact of serum BAFF, TACI, BAFF and APRIL levels on prognosis of B-CLL.

Methods: A total of 129 newly diagnosed CLL patients [median age: 64 (39-88); M/F: 85/44] and 26 healthy volunteers were enrolled in this study. Serum BCMA, TACI, BAFF and APRIL levels were measured at diagnosis using enzyme-linked immunosorbent assay (ELISA). The association with conventional prognostic markers and impact on survival were evaluated.

Results: Serum BAFF, TACI and BCMA levels were significantly lower in the patient group (p<0.05) (Table 1). Serum BAFF ([p=0.008; r=0.236]) and BCMA ([p=0.042; r=0.183]) levels were negatively correlated with Rai stage and serum BAFF level was higher in low-risk patients based on modified Rai staging system (p=0.059). Serum APRIL level was higher in CD38 positive patients (p=0.06, 0.27 (0.0, 1.086) vs 0.13 (0.1, 1.07)). Age (p=0.002), Rai stage (p=0.05) and Modified Rai stage (p=0.051) were the significant factors which had an impact on overall survival in multivariate analysis.

Summary/Conclusions: As BAFF and APRIL display their main biological effects once they bind to their receptors and pass through the intracellular compartment, we consider that it may be more feasible to measure the intracellular levels of these molecules which may be more predictive for B-CLL prognosis. The association of TACI and CD38 expression may indicate the notable balance between proliferation and apoptosis, as CD38 is considered to be a proliferation marker in B-CLL. Further large and prospective studies analyzing the intracellular levels of these molecules are essential to validate the prognostic role of these particular biomarkers in CLL.

PB1797
EXPERIENCE OF IBRUTINIB IN RELAPSED/REFRACTORY B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA AND MANTLE CELL LYMPHOMA IN THE DISTRICT GENERAL HOSPITAL IN A U.K. DISTRICT GENERAL HOSPITAL

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1 Haematology, Derby Teaching Hospitals NHS Foundation Trust, Derby, United Kingdom

Background: The specific determining factors for malignant progression in Chronic lymphocytic leukemia (CLL), remaining unknown.

Aims: To investigate the potential existence of unique cytogenetic profiles associated with specific IGHV repertoires that could be associated with an increased risk of progression in CLL.

Methods: For this purpose, molecular analysis of well-established cytogenetic alterations of chromosomes 11, 12, 13, 14 and 17 together with the pattern of rearrangement of the IGHV genes were performed in 100 CLL cases.

Summary/Conclusions: As BAFF and APRIL display their main biological effects once they bind to their receptors and pass through the intracellular compartment, we consider that it may be more feasible to measure the intracellular levels of these molecules which may be more predictive for B-CLL prognosis. The association of TACI and CD38 expression may indicate the notable balance between proliferation and apoptosis, as CD38 is considered to be a proliferation marker in B-CLL. Further large and prospective studies analyzing the intracellular levels of these molecules are essential to validate the prognostic role of these particular biomarkers in CLL.
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 the time of ibrutinib initiation was 6 months in B-CLL and 6.5 months in 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 who required 2 or more lines of prior 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 who with and without p/53 deletion. 11/26 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 survival. Overall survival. Moreover, patients who received fewer lines of prior treatment had a better survival. Patients with p15/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.

PB1799
THE VALUE OF RITUXIMAB ADDITION TO CHEMOTHERAPY TREATMENT OF REAL-WORLD CLL PATIENTS: A 15 YEAR SINGLE CENTER EXPERIENCE
L. Van Der Straten1, A. G. Dimmohamed2,3, J. K. Doorduijn2, P. E. W. Westerweel1, A. W. Langerak2, A. P. Kater3, M. D. Levin1
1Institute for Hematology, Medical Military Academy; 2Haematology, Erasmus MC Cancer Institute; 3Public Health, Erasmus University Medical Center, Rotterdam, The Netherlands

Background: The addition of the monoclonal antibody rituximab to chemotherapy has been shown to improve progression free survival and overall survival in prospective trials in CLL patients. However, CLL patients participating in clinical trials may not be fully representative of the overall patient population in clinical practice as there is selection bias due to study eligibility. Furthermore, CLL patients may have various concurrent malignancies.

Aims: The aim of the study is to evaluate clinical, laboratory and histopathological features of patients with RS at transformation, and their impact on the outcome.

Methods: We processed data from the medical records of 36 CLL and SLL patients with RS diagnosed and treated in four institutions in Serbia from 2003 to 2016: Clinic for Hematology, Clinical Center of Serbia; Clinic for Hematology, Clinical Center Kragujevac; Clinic of Internal Medicine, Clinical Hospital Center Zemun; and Clinic for Hematology, Medical Military Academy, Belgrade, Serbia.

Results: In 4 institutions RS was diagnosed in 36/1250 CLL/SLL patients (2.8%). Median age was 57.5 years (range 41-79). In 16 (44%) patients RS was confirmed in lymph node sample, in 13 (35%) patients in bone marrow, in 4 (11%) patients in Waldeyer’s ring, in 2 (5%) patients in maxillary sinus, in 2 (5%) patients in liver or/and spleen, while in 3 patients in more than one location. In 11 patients laboratory findings of all patients in transformation were consistent with DLBCL, except one, showing pattern of HL. Prior to the transformation, 26 (72%) patients received chemotherapy (Chlorambucil 6 patients, Fludarabin based regimens 11 patients, CHOP 3 patients, COP/RCOP 4 patients, other modality 3 patients), 4 (11%) of them were on the ‘watch and wait’ strategy, while 1 (3%) patients were on palliative care due to chemotherapy refractoriness.

Summary: The overall survival in patients with RS at transformation was 36 (0-180) months, At the time of transformation median LDH and beta-2 microglobulin levels were significantly higher than on presentation (p=0.035 and p=0.010, respectively). The majority of patients received CHOP (20/36, 55%) and RCHOP (7/36, 19%) as initial therapy. The effects of rituximab (R) addition to chemotherapy showed that the use of rituximab in the first line treatment was significantly associated with improved median survival compared to patients treated with R-fludarabine or R-fludarabine plus cyclophosphamide, 52 (95%) vs 25 (28%) (p=0.030, HR=0.50; 95% CI 0.27-0.93).

Conclusion: The use of rituximab in the first line treatment has significantly improved the median survival. The use of rituximab should be considered to be a part of the standard first line treatment in patients with RS.
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.

**PB1801**

**MONOCONAL B-CELL LYMPHOCYTOSIS AND PROSTATE CANCER: AN UNEXPECTED, POSSIBLE ASSOCIATION**

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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 leukemia (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. MBL incidence varies from 1 to 7% which contained, inter alia, purine analogues, and the monoclonal B-cell population lower than 5000/µl, in the absence of any type of clinical features. In both groups 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.

**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/CD20 PE-Cy7/CD73 APC; Kappa FITC/Lambda PE/CD19 PerCP-Cy5.5/CD20 PE-Cy7/CD5 APC/CD45 APC-Cy7. For each sample, 100000 events were collected. CD5+ 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+ CD20dim selected population was analyzed for light chain clonality and CD22 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 (5.8%). In contrast, in healthy subject group, only one “low-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.**

<table>
<thead>
<tr>
<th>MBL (n. %)</th>
<th>Absolute MBL clone values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>B-cells</td>
<td>B-cells</td>
</tr>
<tr>
<td>(8 cells/µl)</td>
<td>(8 cells/µl)</td>
</tr>
<tr>
<td>3.8%</td>
<td>2.01</td>
</tr>
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</table>

**Summary/Conclusions:** The preliminary results of our prospective study, performed using a routine, not highly sensitive flow cytometry approach, highlight a possible association between (clonal?) MBL and PC, never described before and probably warranting further investigation in a larger number of patients.

---

**Figure 1.**

**Summary/Conclusions:** Incidence of RS in our study is partly coherent with literature data. Levels of LDH and Hb at the time of transition are significant predictors of outcome for patients with RS. Real number of patients with RS is probably higher, but commonly bad condition of these patients on diagnosis of RS probably influences the decision of a clinician not to indicate biopsy.
Chronic myeloid leukemia - Biology

PB1802
IDENTIFICATION OF NOVEL MUTATIONS IN CANCER-RELATED GENES IN HUMAN ERYTHELEUKAEMIA 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 chromosomal translocation t(9;22)(q34;q11), resulting in the formation of the BCR-ABL fusion oncogene. One of the most used CML in vitro model in the K562 cell line, a positive human erythroleukemia cell line derived from a female patient with CML in blastic phase of CML represented by the K562 cell line. 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 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 ESP500 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 sequencings. We confirmed with Sanger sequencing. FISH was performed using commercially available probes (Vysis, Abbott, USA), that identifies BCR-ABL1/fusion genes.

Results: Sequencing and bioinformatic 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>SCLC Reference</th>
</tr>
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<tbody>
<tr>
<td>TP53 136fs*13</td>
<td>NM_001261442.2</td>
</tr>
<tr>
<td>ASXL1 p.Gly53*</td>
<td>NM_03359.5</td>
</tr>
<tr>
<td>AKT1 p.Glu174*</td>
<td>NM_006450.3</td>
</tr>
<tr>
<td>BCR-ABL1 p.Leu419*</td>
<td>NM_0320628.1</td>
</tr>
<tr>
<td>BCORLC1 p.Glu408*</td>
<td>NM_0320628.3</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 LEUKAEMIA
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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, has 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. Up-regulation of BCR-ABL gene at the level of 15–65% were involved. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol-chloroform method. Genotyping was performed by the PCR-RFLP technique.

Results: Combination of genotypes affecting miR-608/BCR-ABL interaction was found to be associated with miR-608 binding site *GG in mature miRNA itself was associated with 81% in CML patients with ineffective 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 TRICOLOR 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 (IFISH) 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 IFISH 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-IFISH 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) 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 cells were seen amongst patients with b2a2 and b3a2 respectively. A mean (± S.D) of 29% (± 30) and 18% (± 17) der(9) deleted cells were 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).

Figure 1.

**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.

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 t(9;22)(q34;q11.2) resulting in the fusion oncogene BCR/ABL in a hemopoietic 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 hematometric 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 specialities (11.5% internal medicine) and 7.7% from the laboratory. Complete blood cell count (CBC) were analysed by ADVA 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 >0.2x10 9/L. A total of 299 requests for PCR of BCR-ABL were reviewed by laboratory hematologists and in case performing the I-FISH classification of Hema-topologic neoplasms criteria. We reviewed clinical history, previous CBC and PB if necessary for this screening. 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-PCRp190 and qRT-PCRp230, 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, monocyteosis, 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 trombocytosis. 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 established as nearly universal in CML 1,3,4, this study reveals it is not always pursue enough, when clinicians ask for a molecular study.

PB1807

**BCR-ABL DEL. C.1086-1270 (R362FS*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 Poulikakos P.I. (2011) and attributed to the formation of heterodimer with “wild type” Bcr-Abl p210 as described by Meggyesi N. (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. (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.

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 TYSROINE 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 oncogene is known to induce high levels of intracellular ROS which may further induce genomic instability with malignant transformation and even resistance to TKI. Variable expression of antioxidant enzymes in leukemia, with limited studies with variable results so far. Altered redox biology in leukemia also has implications for therapeutics.

Aims: We investigated the roles of PRX II in CML primary cells at diagnosis and remission during signal transduction inhibitor (STIs), 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 contributes to find a new pathway on which TKIs are working besides the mechanisms of ATP binding competitively, blocking the binding of ABL-BCR kinase and substrate resulting apoptosis of Ph+ cells. In addition develop the new strategies to overcome the situation of the IM resistance in CML patients, might be a potential target for treatment of CML 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 granulopoietic, megakaryopoietic and erythroid hematopoiesis germs in patients bone marrow. Currently we don’t have definite 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 vitro 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 interleukin-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 for colonies, allowing cytokines and growth factors of mouse body affect human mononuclear cells. It was established that the acquisition of leukemic clone cells 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 the 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 (e1a2b and in 3a2b) 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 pathogenesis.

Aims: To develop a new multiplex RT-PCR method coupled to fragment analysis by capillary electrophoresis to identify different BCR-ABL isoforms: e13a3, e1a2a, e1a4a, e6a2, e1a3, e1a32, e14a2, e1a2, and e8a1.

Methods: 34 CML patients BCR-ABL positive by qRT-PCR and 1 negative control for the qRT-PCR for BCR-ABL were included in this study. The BCR-ABL gene quantification was done using the TaqMan assay with TaqMan probes. These probes were designed for the detection of e1a2b (detection 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 e1a4b and e1a32 isoforms, being in all these cases the e14a2 isoform mainly expressed.

Summary/Conclusions: RT-PCR combined with capillary electrophoresis is recommended as a gold standard 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 (e1a4a 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 t(9;22)(q34.1;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 neutrophin-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 growth and survival of neoplastic cells of the malignant 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 noticed that PDGF-AA level for newly diagnosed CML patients was higher 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 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 detectable patterns of protein expression 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 individual patients, which will be the focus of this group in the future. 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 LEUKEMIA WITH LATE DISCOVERY OF JAK2

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Background: Myeloproliferative neoplasms (MPN) include on the one hand chronic myeloid leukemia defined by the presence of Philadelphia chromosome and BCR-ABL remodelling, and on the other hand MPNs 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 recommends making a point mutation which would include BCR-ABL and V617F JAK2+CMO. However, 28 of those cases were described in a 2013 literature review (3). Most patients developed either a V617F JAK2 mutation during treatment by tyrosine kinase for a BCR-ABL+CMO, a BCR-ABL+CMO 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 leukemia 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 Nilotinib was initiated in 2012 allowing for, to this day, good molecular response despite poor digestive tolerance in the form of dyspepsia. Ever since 2012, the patient has had polycythemia (Hb: 16.7–19 g/dL) that were first attributed to hemocoagulation and inflammation due to recurring bacterial urinary tract infections. Neither infiltration of the lymph nodes nor organomegaly had been noted. In 2014, the patient complained of abundant sweating in the absence of physical effort and, despite the ongoing complete molecular response, hyperleukocytosis was observed (see Figure 1A). In 2015 the patient, then aged 68, signed weight loss of 10 kg despite decent overall state of health. Tomodensitometry found evidence of hepatosplenomegaly. Taking into account the symptoms and persisting blood count abnormalities (WBC 27 G/L, Hb 182 g/L, Platelets 479 G/L, Neutrophils 22.4 G/L, erythrocytosis), a second MPN was suspected. V617F JAK2 mutation was found positive and treatment by Hydrea for essential thrombocythemia was initiated. Adaptation of Nilotinib posology was decided to avoid possible cytopenia due to its association with Hydrea.

Histological examination: (see Figure 1B) As of the last follow-up consultation in 2017, BCR-ABL remains undetectable and the overall state of health was preserved. Hyperleukocytosis as well as myelerythemia were persistent on the 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 as 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 support to the second, but this requires a larger study group 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.
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 MR3 after 7 and 12 months, 1 patient is not yet evaluable. Univariate analysis showed no difference in relapse risk according to age, gender, type and duration of TKI, duration of stable DMR and sokal score risk. 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 (78%) maintained DMR vs 4/6 e13a2 patients (67%) (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.
Summary/Conclusions: In e14a2 CML patients the probability of discontinuation, or stable 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 IMITINIB VS SUSTAINING STANDARD-DOSE IMATINIB IN PATIENTS WITH CP CHRONIC MYELOID LEUKAEMIA FOLLOWING OPTIMAL MOLECULAR 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 75%-90% of imatinib-treated patients are in sustained complete cytogenetic remission (CCyR) and undetectable molecular residual disease (UMRD) rates by 36 months. In this study, we investigated the efficacy of nilotinib (NIL) versus high-dose IM versus sustaining standard-dose IM for the patients in CCyR with suboptimal molecular response to first-line IM therapy.

Methods: Early CP CML patients who have achieved CCyR but not MMR after 18 to 24 months on first-line IM therapy at a daily dose of 400 mg were divided into the three treatment groups; nilotinib (NIL) 400mg BID (800 mg/day; group 1) vs IM 400 mg BID (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 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, 12-96), and 36 months (range, 12-72) in each group, respectively. All patients in group 1 remained NIL treated, 18 patients in group 2 crossed over to NIL 400mg BID due to intolerance (n=4) and lack of response (n=14), in group 3, 22 patients switched to other treatment due to intolerance (n=7), lack of response (n=1), failure (n=1), or treatment-related death (n=2) and 12 patients lost to follow-up. When data on patients who crossed over to the other treatment was included, cumulative incidence (CI) of MMR by 36 months was significantly higher in group 1 than group 2 (83.1% vs 57.1%, P=0.021), but there was no difference in group 1 vs 2 (P=0.195) and group 2 vs 3 (P=0.297). CI of MRF-3 by 36 months showed a trend of higher in group 1 than the other two group (11.7% vs 0% vs 2.6%, group 1 vs 2, P=0.066, group 1 vs 3, P=0.099, group 2 vs 3, P=0.405).

Summary/Conclusions: NIL 400mg twice daily treatment showed better efficacy than standard-dose IM for the treatment of patients who have suboptimal molecular response to first-line IM. Additionally, a switch to NIL in suboptimal molecular responder to IM had a trend for achieving a MMR more frequently, suggesting the potential benefit of a treatment-free remission.

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 3 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=0.887), and these values were higher than that in the newly diagnosed CML patients (19.0mmHg), though without significance (p=0.38). Nine 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.

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 TRPG 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 TRPG 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.

PB1817

DYNAMICS OF BCR-ABL1 MUTATION ACQUISITION AND LONG-TIME MUTATION-ASSOCIATED RESISTANCE PROGNOSIS IN PATIENTS WITH CP CHRONIC MYELOID LEUKAEMIA TREATED BY TYROSINE KINASE INHIBITORS: RUSSIA, 2006-2016

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Background: While chronic myeloid leukemia (CML) can successfully be treated with tyrosine kinase inhibitors (TKIs), mutations in the BCR-ABL1 kinase domain are the most prevalent cause of TKI resistance. More than 100 BCR-ABL1 kinase domain point mutations with various frequencies of incidence, domain positions and implications on TKI response in CML are associated with
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 hospital centers in Russia with 1241 cases 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 TKI resistant mutations. The frequency of each mutation varied from 0.01-4.9%. The most frequent mutations were associated with decreased response to the TKI therapy. The frequency of imatinib-resistant mutations decreased gradually from 2006 to 2016, while the rate of F317L and F359V mutations underlying resistance to second generation TKI increased in 2013-2016. T315I mutation rate expanded to the maximal level in 2014 and abruptly decreased afterwards. This tendency change may be the consequence of the second generation TKIs and other therapeutic strategies involvement into clinical practice.

Summary/Conclusions: As different as 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 the early detection of resistance. We believe here that 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 LEUKAEMIA TREATED FRONTLINE WITH NILOTINIB

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Aims: To define the starting dose for the randomized phase II study which tested the efficacy of nilotinib (NIL) in newly diagnosed adult CML patients treated frontline with nilotinib (NIL).

Methods: An analysis of 345 CML patients at diagnosis (chronic phase) enrolled within 3 multicentric prospective studies of the GIMEMA CML Working Party (NCT00481052, NCT00769327, NCT01535391) was performed. The initial treatment was NIL 300 mg Bid or NIL 400 mg Bid. Definitions: major molecular response (MMR), BCR-ABL1 IS ratio <0.1%; deep molecular response (MRD<0.1%) with >10,000 ABL1 copies; progression, transformation to advanced phases; death, at any time and for any reason. Cumulative incidences of response were estimated under consideration of competing risks (progression, death) and compared by Gray test. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method and compared by log-rank test.

Results: Patients expressing rare transcripts (e12a2 or e19a2; n=7) and patients with unknown transcript type (n=10) were excluded: 328 patients were evaluable: 89% e13a2 transcript, 53% with e14a2 transcript and 3% expressing both transcripts. No significant differences in age, gender, Sokal or EUTOS long-term survival score distribution, presence of clonal chromosomal abnormalities in Ph+ cells, or NIL dose were observed. The median follow-up was 60 months (range 24-82 months). The response rates and the survival probabilities were similar in patients with e12a2 transcript and other transcripts. The rate of patients with e14a2 transcript (N=174), but the differences were not significant: MMR by 12 months, 66% vs 72%, p=0.244; MRD<0.1% by 36 months, 56% vs 66%, p=0.067; estimated cumulative incidence of MMR, 82% vs 88%, p=0.135; estimated cumulative incidence of MRD<0.1%, 60% vs 69%, p=0.101; estimated PFS, 88% vs 93%, p=0.547; estimated OS, 95% vs 94%, p=0.904. 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 both groups with e13a2 alone (N=244), no significant differences were significant (cumulative incidence of MMR and MRD<0.1%, p=0.050 and p=0.038, respectively), but no outcome differences emerged (PFS and OS, p=0.340 and p=0.276, respectively).

Summary/Conclusions: Lower molecular response rates in patients with a rare transcript that were obtained in the differences were small and mostly not significant. No outcome differences were detected. Further studies in larger patient cohorts are required in order to clarify whether including the transcript type in the calculation of the baseline risk scores may improve prognostic stratification, and whether NIL or other second generation TKIs should be preferred as first-line therapy in patients aiming at treatment-free remission.
Three patients (2 IM/HU, 1 IM) were lost to follow-up. As prospectively designed, all available IM/HU patients (n=77) were included. 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 equal 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. With IM/HU, the probabilities 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<0.001). The 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 rates were 41.1 (p=0.0383) 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 groups 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< 0.00343, Gray test).

**Summary/Conclusions:** Compared to imatinib only, the combination of imatinib 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.

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**PB1820**

A MULTICENTER, OBSERVATIONAL, AMBISPECTIVE STUDY EVALUATING EFFICACY AND SAFETY OF GENERIC IMATINIB COMPARED TO GLEEVEC IN CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE - 3 MONTHS RESPONSE ANALYSIS

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**Methods:**

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.

**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 was detected at diagnosis in 20 (67%) patients, 3 (10%) patients were initially treated with Nilotinib. 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, 11q23(22)(q12;q22);(q34;q11);(17)(q10), trisomy 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.

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**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 was detected at diagnosis in 20 (67%) patients, 3 (10%) patients were initially treated with Nilotinib. 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, 11q23(22)(q12;q22);(q34;q11);(17)(q10), trisomy 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).

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**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.
PB1822

BCR/ABL1 TRANSCRIPTIONAL ANALYSIS OF 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

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Background: Several types of transcripts can be produced during chromosomal translocation, which lead to the formation of the BCR/ABL fusion gene in patients with chronic myeloid leukemia (CML). Previous results of a few large studies showed that patients with CML in chronic phase (CP) with e13a2 transcript have inferior responses to frontline imatinib therapy compared to patients with the e14a2 transcript.

Aims: To investigate the prognostic significance of e13a2 and b14a2 BCR/ABL1 transcripts in CML patients switched to nilotinib after suboptimal response or failure on frontline imatinib.

Methods: CP-CML patients (N=143) who did not achieve complete cytogenetic response (CCR) after imatinib therapy (600 or 800 mg once daily) and were switched to nilotinib 400 mg twice daily, were enrolled in present study (55 patients with e13a2 transcript and 88 patients — with e14a2 transcript). The primary and secondary resistance before switching to 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 co-expression were excluded from the analysis. Probability of overall survival (OS), progression-free survival (PFS), and event-free survival (EFS) were 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, x2-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 Ph-positive cells. No correlation of transcript type with age or sex was observed. Transcript e13a2 was associated with higher WBC (120x10⁹/L vs. 95.3x10⁹/L, p=0.02) and lower baseline percentage of eosinophils (p=0.041). No differences were found in other differential counts of peripheral blood, hemoglobin concentration, or spleen size. The time to CCR, MMR and MR4.0 and rate of CCR (52% and 52%), MMR (38% and 33%) and MR4 (23% and 22%) were comparable in patients with e13a2 and e14a2 transcripts respectively. Estimated probability of CCR, MMR and MR4.0 also did not differ in both groups. The rate of optimal response, primary and secondary resistance before switching to nilotinib was comparable in both groups. Whereas there were no differences in the estimated probability of CCR loss in both groups, but rate and cumulative incidence of MMR loss was significant higher (69% vs. 11%, p=0.037) in patients with e13a2 transcript. No difference between groups was observed with regard to PFS, EFS and OS. Summary/Conclusions: Analysis of 143 CML patients treated with nilotinib as the 2-nd line therapy suggests that patients with e13a2 transcript have less stable therapy response and demonstrate higher cumulative incidence of MMR loss (molecular relapse). But outcome differences were not observed. Further analysis of a larger number of events and longer observation is required.

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

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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. HRQoL profiles based on de-novo treatment response 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: 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 (warming 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 limitation limitations in the emotional health problems (P =0.0019) and role limitations due to emotional problems (P =0.0011) 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 physical problems (P =0.0404). Female gender, and higher education level were also associated with better HRQoL subscales.

Summary/Conclusions: Achieving optimal response at 12 months was associated not only with longer overall survival and less treatment failure and disease progression, but also better HRQoL in newly diagnosed patients with CML-CP on front-line tyrosine kinase inhibitor.
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-on (2L).

Aims:This study aims to investigate time to achieving MR4.5 and major molecular response (MMR; ≥3-log reduction or ≤0.1% in BCR-ABL1 on IS in CML-CP patients (pts) treated with nilotinib vs dasatinib in 2L.

Methods:In this cross-sectional panel approach, 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 1/1/15, and had ≥12 mos of follow-up data after initiating 1L TKI. Multivariable Cox proportional hazards models accounting for county 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, 1L vs 2L generation TKI as 1L, and reasons for 1L TKI discontinuation. Adjusted hazard ratios (HR) and 95% confidence intervals (CI) 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, median age at 1L was 54 years, and were 35% female. 42% of 1L nilotinib and 1L dasatinib pts were treated with the other 2nd generation TKI in 1L (p<0.01). A higher proportion of nilotinib pts had high-risk Sokal score (20.9% vs 11.6%, p=0.05) and received prior hydroxyurea (8.7% vs 3.3%, p=0.08) vs dasatinib. 85% and 11% of 2L nilotinib pts discontinued 1L TKI due to resistance and intolerance, respectively, prior to switching to nilotinib, vs 74% and 22% for 2L dasatinib pts (both p<0.05). The univariate Cox model showed that nilotinib had a non-significantly higher rate of achieving MR4.5 than dasatinib (32% vs 31% at 24 mos for 2L nilotinib and 2L dasatinib, respectively, based on the Kaplan Meier estimator; unadjusted HR=1.09, 95% CI [0.87, 1.38], p=0.46); however, after multivariate adjustment, nilotinib reached a significantly higher rate of achieving MR4.5 (adjusted HR=1.36, 95% CI [1.07, 1.73], p=0.01) than dasatinib. Among those who achieved MR4.5, 45% of nilotinib pts maintained MR4.5 for ≥1 y vs 39% of dasatinib pts (p=0.60). Additionally, high-risk Sokal score (HR=0.31, 95% CI [0.14, 0.72], p<0.01) and resistance to 1L TKI (HR=0.60, 95% CI [0.24, 0.88], p=0.01) were inversely associated with achieving MR4.5.

There was no significant difference in MMR achievement between 2L TKI groups. Over 3 times more dasatinib pts experienced pleural and pericardial effusion AEs than nilotinib pts (9.9% vs 2.6%, p<0.02). One nilotinib pt had ischeamtic heart disease AE vs none for the dasatinib group (p=0.49).

Summary/Conclusions:This retrospective chart audit study suggests that 2L nilotinib may be associated with a higher rate of MR4.5 than 2L dasatinib in CML-CP. Our results should be taken with caution as this study is susceptible to unmeasured confounding and biases due to its retrospective and observational nature. Rigorous clinical assessment in a prospective setting is needed to conclusively rates of patients achieving MR4.5.

PB1826

COMPUTATIONALLY INTELLIGENT PREDICTION OF CLINICAL OUTCOME IN CHRONIC MYELOID LEUKEMIA

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Background:Computational intelligence has been applied to a wide range of problems to assist in decision-making, especially artificial neural networks, fuzzy systems and powerful hybrid neuro-fuzzy approaches have already proven their strong potentials in medicine. Despite that, applications in hematology are still scarce.

Aims:In this study we have developed novel ANFIS neuro-fuzzy prognostic models based on morphometric and morphometric diagnostic data, to enable better prediction of complete cytogenetic response (CCgR) for patients with chronic myeloid leukemia.

Methods:This prospective study included a consecutive series of patients with chronic myeloid leukemia (CML) who were started on imatinib therapy. Analysis was made from 1C to 3A phases, with 12 and 18 months as the outcome variables. A total of 40 patients on imatinib therapy were included in the final analysis. Of these, 25 (62.5%), 29 (72.5%), and 32 (80%), respectively, achieved CCgR at 6, 12, and 18 months after initiation of imatinib. Computationally intelligent neuro-fuzzy models that were developed included EUTOS score on diagnosis and one of the following morphometric parameters: microvascular density, length of the minor axis, area or circularity of the blood vessel. Adaptive neuro-fuzzy systems represent a specific combination of artificial neural networks and fuzzy logic, thus combining the learning ability of artificial neural networks with the knowledge representation capability of fuzzy logic systems. ANFIS (Adaptive Neuro Fuzzy Inference System) consists of five layers of nodes (neurons), each of which performs a particular function on incoming signals as well as a set of parameters pertaining to this node. The basic architecture of ANFIS using hybrid learning algorithm is presented in Figure 1.

Results:All analysed patients have received imatinib mesylate as their first-line treatment for CML. Model predictions (0–1) for any individual patient were interpreted as probability of CCgR at 6, 12 or 18 months. The overall accuracy of the final model was determined by comparing the predicted values with the actual events. A probability cut-off point of 0.50 (50%) was used to classify observations as events or non events, and patients were divided in training, validation and testing groups. Best performing of ANFIS model, including EUTOS score and minor axis morphometric parameter was better than a model that includes only EUTOS score and regression model based on the same inputs. Overall model correct classification achieved for EUTOS, two input LR model and two input ANFIS model were respectively 75%, 75% and 75.5%, while areas under curve on ROC graphs were 0.776, 0.829 and 0.875 respectively.

Figure 1.

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.
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 blastic (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. Response assessment: Response assessment was 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.

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 distinctive 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 cases and the number of reported cases is rising. The 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(+) 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, cytogenetics 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 x10^9 (range 6-36) and platelet count - 1316 x10^9 (range 770-2815). Spleenomegaly was found in 5 pts (16.1%). 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(+) b3a2a2 (n=16) or b2a2 (n=15). Karyotypes were available in 13 pts and commonly classical Ph chromosome was found in 16 of them (69%), with inv(9) (21.7%) a cryptic translocation was detected 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% (n=8), while 5 were switched to a second line, and 2 - to a third line therapy. First-line treatment with nilotinib in 11 pts resulted in optimal response in 80% of them (n=8). In 10 pts the optimal molecular response (MR) was achieved in 80% (n=20), including deep MR in 56% (n=14). 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 proportion surviving at 5 years was 91%.

Summary/Conclusions: Interestingly, CML presenting with isolated thrombocytopsis 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.

BCR-ABL1 MOLECULAR RESPONSES AT 12-18 MONTHS USING THE QUANTIDEX qPCR BCR-ABL1 IS KIT PREDICT LONG-TERM EVENT-FREE SURVIVAL IN PATIENTS WITH TKI-TREATED CML

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Background: Detection of BCR-ABL1 e13a2 or e14a2 transcripts (major break- point) in BCR-ABL1 fusion transcripts (also known as the Philadelphia chromosome) is important in CML monitoring tumor burden. The International Scale (IS) was established to standardize reporting relative to a common baseline. As newer TKI therapies create deeper responses, analytical sensitivity has become a critical topic in investigations into TKI discontinuation, where researchers require a clinically validated assay that shows a molecular reduction (MR) of ≥4-5 logs below baseline (i.e. MR4.5 or 0.0032%IS).

Aims: To clinically validate the QuantideX qPCR BCR-ABL1 IS Kit and to reaffirm the clinical utility of BCR-ABL1 RT-qPCR monitoring in patients with t(9;22) positive CML, a correlation between molecular response (MR) values and long-term outcome was determined.

Methods: The QuantideX qPCR BCR-ABL1 IS Kit uses standard TaqMan chemistry to quantitate BCR-ABL1 and the ABL1 reference gene. Associated software reports an international scale BCR-ABL1 value and a log-transformed MR value, with a 3-log-reduction from pre-treatment baseline representing 0.1%IS or MR3.0. Three laboratories performed BCR-ABL1 testing on banked RNA specimens from 96 chronic phase CML patients from 2 hospitals drawn 12-18 months after starting TKI therapy. Clinical events (TKI therapy change, loss of complete hematologic or cytogenetic response, progression to accelerated phase or blast crisis, kinase domain mutation, or death) were recorded through 36±4 months after starting TKI. Two operators per site also tested serially-diluted reproducibility samples (range MR1.0 to MR4.0) in multiple replicates over 5 days. The 95% LOD for the assay was defined as the median measured%IS value of 4 analogous serially-diluted specimens.

Results: 51 patients had MR >3.0 at 12-18 months post-TKI. Of these 51 patients who did not achieve a major molecular response (MMR), 20 had a subsequent clinical event, 17 had no event, and 14 were lost to follow-up (LFU). 45 patients had MR≥3 at 12-18 months post-TKI. Of these 45 pts who did achieve MMR, 8 had an event, 28 had no event, and 9 were LFU. Kaplan-Meier survival curves demonstrated a 22% prolongation of event-free survival (95% CI 2%>42%) at 3 years between the two MR groups [p=0.028; 58% (95% CI 44%-75%) for MR3 vs 80% (95% CI 68%>93%) for MR4]. Specimens with MR values ranging from MR1.0 to MR4.0 in multiple replicates over 5 days. The 95% LOD for the assay was defined as the median measured%IS value of 4 analogous serially-diluted specimens.

Conclusions: 73% of patients had MR >3.0 at 12-18 months post-TKI. Of these 51 patients who did not achieve a major molecular response (MMR), 20 had a subsequent clinical event, 17 had no event, and 14 were lost to follow-up (LFU). 45 patients had MR≥3 at 12-18 months post-TKI. Of these 45 pts who did achieve MMR, 8 had an event, 28 had no event, and 9 were LFU. Kaplan-Meier survival curves demonstrated a 22% prolongation of event-free survival (95% CI 2%>42%) at 3 years between the two MR groups [p=0.028; 58% (95% CI 44%-75%) for MR3 vs 80% (95% CI 68%>93%) for MR4]. Specimens with MR values ranging from MR1.0 to MR4.0 in multiple replicates over 5 days. The 95% LOD for the assay was defined as the median measured%IS value of 4 analogous serially-diluted specimens.
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 signs; 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 60% 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: 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. Patient selection criteria for initial TKI treatment are changing, especially if TKIs are approved for second-line treatment. Therefore, 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 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. A total of 87 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 (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, leucocyte 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), leukocytosis100x109/L (HR=3.158; 1 point), blasts in peripheral blood ≥1% (HR=2.912; 1 point), and presence of additional cytogenetic aberrations (ACA) (p=0.002) as a predictors of Imatinib failure. In agreement, we assigned risk scores based on hazard ratios (HR) to hepatomegaly (HR=4.089; 2 points), leukocytosis >100x109/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% (30/86) in intermediate and 76.7% (23/30) in high risk group (HR=3.973, 96% CI for HR 2.237-7.053, 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 guideline 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 aim of this audit was to determine the impact of TKIs on symptom burden and QOL in patients currently on TKIs 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 (MDASI) tool.
Results: 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 (MDASI) tool.
Results: Of the 87 patients surveyed, the most commonly prevailing symptoms were fatigue (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 symptom prevalence or severity among the different TKIs. As regards the perceived interference of symptoms on daily functioning, only 29% reported a score of 7 or more in at least 1 of the 6 interference items (i.e. general activity, mood, work, relations with others, walking and enjoyment of life), and only 14% reported that their enjoyment of life was severely affected (score of 7 or more). Of note, exactly two thirds of patients reported little or no interference with their enjoyment of life (score of 0-3).

Summary/Conclusions: As demonstrated in this audit, patients with CML on TKI therapy experience chronic tolerance with other TKIs. Only patients taking second generation TKIs did not appear to have any difference in frequency or severity of symptoms or in QOL compared to patients imatinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential for adverse events with long term therapy may result in dose adjustments, treatment discontinuation, or nonadherence, all of which may negatively affect treatment efficacy. Therefore, assessment of QOL and the symptom burden experienced by patients with CML is useful to facilitate individual treatment decisions and to improve outcome as well as to evaluate the efficacy of emerging therapies.

PB1833

COST-EFFECTIVENESS OF A THERAPEUTIC EDUCATION PROGRAM (TPE) FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND TREATED BY TYROSINE KINASE INHIBITORS

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Aims: Within our cancer centre, an TPE program on ITK in the management of CML has been authorized since 2011. We conducted a pharmacoeconomic study to evaluate the TPE clinical impact on responses to TKI in patients with CML (based on recommendations from European Leukemia Net) and also the costs in terms of use of care.

Methods: Over the 12-month follow-up period, the study population consisted of 2 groups of CML patients monitored in our centre: - Intervention group (n=18) (IG): Patients who benefited of TPE sessions on TKI between January 2013 and August 2015 - Matched controls’ group (n=18) (CG): Patients who benefited only from the usual care, matched to the “intervention” group. The method of pairing the 2 groups of patients according to the age at diagnosis, sex, the molecule used in first line and the prognostic risk according to the score of Sokal was used. The main criterion of efficacy was the MMR. The considered costs were: the cost of the TPE program, estimated on the basis of the French health insurance reimbursement per patient and the costs associated with the use of “supplementary” care (examinations, consultations and additional hospitalizations). The point of view was from French health insurance.

Results: Over the 12-month follow-up period, the number of patients in MMR was similar between the 2 groups (9 in IG versus 8 in CG). However, the average time to obtain the MMR was significantly shorter in IG (6.9 months vs 3.8) than in CG (11.3 months vs 2.1) (p <0.05). The mean duration of MMR maintenance over the 12-month follow-up period was significantly longer in IG (3.2 months vs 0.5) than in CG (1.5 months vs 1.9) (p <0.05). Regarding the use of additional care, unexpected hospitalizations were significantly more numerous in CG than in IG (4 versus 0). Thus, costs associated with use of additional care were significantly lower in IG (€ 3,566) than in CG (€ 12,709). Thus, € 250 invested (annual allowance per patient) in the TPE saves € 508 in the use of care and reduces the time required to obtain a MMR by 4.4 months.

Summary/Conclusions: Thus, TPE is clinically and economically beneficial in our study population. By increasing the patient capacity to adapt to the treatment through the development of skills and adaptation processes, TPE reduces the costs of seeking care while improving the clinical response to treatment with a faster and more sustainable major molecular response.

PB1834

ROLE OF ALLO-HSCT IN THE TREATMENT OF PATIENTS WITH T315I MUTATION IN THE TKI ERA

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Aims: To evaluate the thyroid functional status in CML patients treated with imatinib and nilotinib.

Background: Resistance to tyrosine kinase inhibitors (TKI) in patients with chronic myeloid leukemia (CML) is frequently caused by point mutations in the BCR-ABL kinase domain, including the gatekeeper mutant T315I, which confers a high degree of resistance to all currently approved tyrosine kinase inhibitors except ponatinib. The role of allo-HSCT in such patients is still disputable.

Methods: Retrospective analysis of 53 BCR-ABL T315I-positive CML patients (pts) was done. Allogeneic bone marrow transplantation (allo-HSCT) was made in 16 pts, 37 pts received only pharmacological therapy (21 pts received TKI as monotherapy or in combination over 16 pts received hydroxyurea, interferon-α or chemotherapy). At the time of T315I detection 29 (55%) pts were in CP, 19 (36%) pts had AP and 5 (9%) pts were in BC. Median (Me) age at the time of mutation detection was 47 years (15-78) (38 years in HSCT-group), 2 pts were in BC at the time of HSCT; 5 pts were in AP, 7 pts were in CP2z. The number of points on EBMT scale: 3-4 points – 12(75%) pts, 5-7 points – 4(25%) pts. 11 (69%) pts received more than 2 lines TKIs before allo-HSCT.

Results: The median follow-up time after T315I detection was 21 months (1-100). 5-years OS in whole group was 42% (Figure 1A). According to multivariate analysis only CML phase at the time of mutation detection significantly affect to survival in whole group. All pts in BC (n=5) in HSCT group and in non-HSCT group died within first year after T315I indication wherein Me survival time was 1.3 month (Figure 1B). 5-years OS in non-HSCT group (n=37) was 42% with Me survival time 2.8 years. 5-years OS after allo-HSCT (n=16) was 37% with Me survival time 5 months (Figure 1C). All living patients after allo-HSCT are in deep molecular response. There was no significant difference in 5-years OS between TKI (n=21) and non-TKI (n=16) pharmacological therapy (non-HSCT) groups (42% and 47% respectively, p=0.53) (Figure 1D).

Figure 1.

Summary/Conclusions: Detection of T315I mutation in TKI-resistant patients is extremely unfavorable factor for survival, especially in the advanced phase CML, and it is a great reason for switching to ponatinib or other new potential investigatory drugs if possible. Allo-HSCT can be a potential option for this group of patients in case of good selection taking into consideration transplant risk, especially for patients in CP 2z.

PB1835

THYROID FUNCTIONAL STATUS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA ON TKIS - SINGLE-CENTER RESULTS

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Aims: To evaluate the thyroid functional status in CML patients treated with imatinib and nilotinib.

Background: Tyrosine kinase inhibitors (TKI) as target specific compounds profoundly changed the outcome in patients with chronic myeloid leukemia (CML). TKI-induced thyroid dysfunction is now recognized as a common toxicity associated with some TKI. In this previous 16 pts received hydroxyurea, interferon-α or chemotherapy. At the time of T315I detection 29 (55%) pts were in CP, 19 (36%) pts had AP and 5 (9%) pts were in BC. Median (Me) age at the time of mutation detection was 47 years (15-78) (38 years in HSCT-group), 2 pts were in BC at the time of HSCT; 5 pts were in AP, 7 pts were in CP2z. The number of points on EBMT scale: 3-4 points – 12(75%) pts, 5-7 points – 4(25%) pts. 11 (69%) pts received more than 2 lines TKIs before allo-HSCT.

Results: The median follow-up time after T315I detection was 21 months (1-100). 5-years OS in whole group was 42% (Figure 1A). According to multivariate analysis only CML phase at the time of mutation detection significantly affect to survival in whole group. All pts in BC (n=5) in HSCT group and in non-HSCT group died within first year after T315I indication wherein Me survival time was 1.3 month (Figure 1B). 5-years OS in non-HSCT group (n=37) was 42% with Me survival time 2.8 years. 5-years OS after allo-HSCT (n=16) was 37% with Me survival time 5 months (Figure 1C). All living patients after allo-HSCT are in deep molecular response. There was no significant difference in 5-years OS between TKI (n=21) and non-TKI (n=16) pharmacological therapy (non-HSCT) groups (42% and 47% respectively, p=0.53) (Figure 1D).

Figure 1.

Summary/Conclusions: Detection of T315I mutation in TKI-resistant patients is extremely unfavorable factor for survival, especially in the advanced phase CML, and it is a great reason for switching to ponatinib or other new potential investigatory drugs if possible. Allo-HSCT can be a potential option for this group of patients in case of good selection taking into consideration transplant risk, especially for patients in CP 2z.
**Methods:** This cross-sectional study comprised 85 patients with CML in chronic phase, treated with imatinib, and one in chronic myeloid leukaemia (CML) treated with Tyrosine Kinase Inhibitors (TKIs) who has relative survival rates of up to 90% that of age-matched controls. Patients achieving complete cytogenetic responses (CCyR) within 2 years of starting imatinib have survival rates equivalent to the general population. Newer TKIs are associated with faster and deeper treatment responses, but have a more toxic side effect profile as well as being more costly.

**Aims:** This study looks at the 11 year experience of a single teaching hospital treating a population of almost one million and presents the response and survival data of this unselected population of patients with CML treated with imatinib as initial therapy.

**Results:** In total 83 patients were newly diagnosed in this time period. Four patients, treated on SPIRIT2 with dasatinib as initial therapy, have been excluded from the subsequent analysis, leaving 79 patients treated initially with imatinib 400mg daily. The median age at diagnosis was 53 years (range 13-95) with 54% of patients. The median follow up was 16 months (range in living patients 29-163 months). Fifteen patients have died (19%). The median age at diagnosis of these was 73 years. Two deaths were transplant-related, both in patients who had failed available TKIs and had mismatched transplants. The other patient who died of accelerated disease was intolerant of all TKIs and unfit for transplant. Three patients died of other malignancies (ovarian, bowel and melanoma). Seven patients were transplanted. Of the surviving 5, 2 had sibling transplants early in the TKI era, 2 had MUD transplants after failing imatinib prior to the availability of second line drugs, and one failed to make an adequate response to imatinib then nilotinib and received a stem cell transplant. A selected population, up front imatinib with appropriate response (BCR-ABL ratio < 0.01, MMR). An MMR was achieved by 60/79 (76%) patients. Of the 19 without MMR, 1 is lost to follow-up, and 9 have died, of which only one death was due to accelerated CML in a patient intolerant of all TKIs. Of those 9 patients living not in MMR, 8 have a CCyR. Three are elderly patients and 5 have taken a pragmatic approach: three are entered to patient compliance, two to treatment limited by severe side effects and one had TKI interruption to facilitate cancer treatment. Of the sixty patients in MMR, 40 achieved this on standard dose imatinib. Four patients required increased dose of imatinib, 11 were switched to second line TKI and 5 were transplanted. A complete molecular response (BCR-ABL ratio < 0.003, CMR) was achieved by 10 patients, six on standard dose imatinib. Nine patients presented with CML in chronic phase, treated with imatinib in the TKI era. At six years follow up, the overall survival was 86% which is remarkably similar to that of the IRIS trial patients. By using an intention to treat analysis, 66% of the overall population achieved a complete cytogenetic response. Clinical experiences (ovarian, bowel and melanoma). Seven patients were transplanted. Of the 149 patients (median age 54.5 years; 57% was males) in chronic phase of CML. The median follow-up from time of diagnosis and start of therapy was 45 months and 39 months, respectively (range 3-145 months). Of the patients who waited less than 6 months was 0 months (range 0-6). In 11 patients, overall survival for patients on frontline imatinib (Group 1) and frontline nilotinib (Group 2) was 83% and 87%, respectively. According to ITT principle, achievement of CCyR and MMR at 24 months was higher in Group 2 compared to Group 1 (81% vs 66% and 74% vs 37%, respectively). Rate of death was similar in both studies (20/118 vs 4/31). When we analysed delayed therapy at 24 months, CCyR for patients who received therapy 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 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 therapy. Achievement of CCyR and MMR at 24 months was higher in patients on frontline nilotinib therapy. Patients who waited for therapy had optimal response regardless the wait period on nilotinib therapy.

**PB1838**

**THE INFLUENCE OF AGE ON TREATMENT OUTCOME OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING FRONLINE IMATINIB**

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**Background:** CML patients in developing world had to wait for the start of TKI treatment, from several months to years. The significant delay in proper treatment of imatinib has had drastic consequences on patient outcomes including 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 frontline imatinib and frontline nilotinib therapy in Bosnia and Herzegovina in the period from 08/2005 to 08/2016, categorized based on delayed start of therapy.

**Methods:** All newly diagnosed CML patients in CML-CP (n=149) who started their TKI treatment in period from August 2005 to August 2016 were included in this multi-centre retrospective analysis. The prevalence of hypothyroidism in our study group did not differ from general population. Additional study on a larger sample size and evaluation of antibodies is required.

**Results:** Of 85 patients in CML-CP (n=149) who started their TKI treatment in period from August 2005 to August 2016 were included in this multi-centre retrospective analysis. The prevalence of hypothyroidism in our study group did not differ from general population. Additional study on a larger sample size and evaluation of antibodies is required.
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-spherocytic 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, 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. 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 cytometry 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.

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 tool for measuring red cell blood 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 diagnostic differentiation 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 osmocsm module of the Laser-assisted Optical Rotational Deformability Cell Analyzer: LoRRCa MaxSis (RR Mechatronics). Evaluation of osmocsm parameters

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 adverse event (AEs), the 5-year event-free survival (EFS) and 5-year overall survival (OS) were all elevated. 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 Sokai scores in the EP group than in the groups MA and YA (p<0.001). To the contrary of that, most patients with high EUOTS 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 rates 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 59.1-78.1)) and in EP group (60.2% (95% CI 49.5-73.7)). The 5-years OS in the EP group (74.7% (95% CI 65.9-89.0)) 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.
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. Statistical analysis was operated with GraphPad Prism.

Results: Specific patterns of osmoscan 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 EImax and AUC. HE curves showed a characteristic trapezoidal shape with a decreased EImax, Omax and AUC. dHSt curve was bell shaped with a specific decrease in Othyper and a slight increase in Elmin. Reference ranges for each osmoscan parameter were established with 171 healthy subjects and compared with 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 HE (sensitivity 94.40%, 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 (sensitivity 95.38% and specificity 99.42%) 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%).

Conclusion: To conclude that, the inclusion of LoRRCa osmoscan 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 dHSt patients.

PB1841
RARE RED BLOOD CELL ENZYMOPATHIES INDUCED CHRONIC NONSPHEROCYTIC HYEMOLYTIC 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. The gene-by-gene approach is expensive, time consuming and cumbersome as testing for multiple genes is required. Molecular diagnosis by gene-by-gene approach is expensive, time consuming and cumbersome as testing for multiple genes is required. Molecular diagnosis by gene-by-gene approach is expensive, time consuming and cumbersome as testing for multiple genes is required. 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 when 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 VariantStudio™ v2.1 were used for analysis, classification, and reporting of genomic 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 re-sequencing 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/ mutatop and Mutatontaster. 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. Definitive diagnosis of the cause in our patient is likely PK deficiency and further investigations are required to confirm this possibility.

PB1842
COMPARISON STUDY OF THE EOSIN-5'-MALEIMIDE BINDING TEST, OSMOTIC FRAGILITY TEST AND CRYOH EMOLYSIS 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 parameters of the fluorescent EMA test 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. Hemoglobin, 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 (ft), MCHC (%) and total bilirubin level were shown in Table 1. Besides MCV HS were below normal range, there were greater degrees of anemia in the HS groups compared to the control 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 (Table 1). There were moderate concordance 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. EMA was superior diagnostic test to osmotic fragility. The sensitivity of cryohemolysis test was 90.48%, specificity was 94.12%, PPV was%88.37, NPV was%95.24.

Table 1. Comparison of Clinical and Laboratory Findings in Hereditary Spherocytosis groups and Healthy Controls

<table>
<thead>
<tr>
<th>Sex</th>
<th>M/F</th>
<th>Age (yr)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>OF</th>
<th>EMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls</td>
<td>38/47</td>
<td>12 (1-20.0)</td>
<td>69.3 (58.9-83.0)</td>
<td>77.2 (69.4-83.0)</td>
<td>76.4 (73.0-80.0)</td>
<td>90.48%</td>
<td>94.12%</td>
</tr>
<tr>
<td>Hereditary Spherocytosis</td>
<td>62/184</td>
<td>11 (1-19.0)</td>
<td>63.6 (51.9-79.9)</td>
<td>74.5 (67.9-80.0)</td>
<td>73.8 (64.8-84.0)</td>
<td>82.5%</td>
<td>88.37%</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 Parallel Analysis of Variance-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 thermogravimetry in conjunction with a mutul-
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 TG7 (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 cells 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 CONSSENSUS 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 in Mediterranean areas. The spectrum of signs and symptoms of the different GD phenotypes ranges from fatal perinatal to asymptomatic adult disease, and the heterogeneity of its presentation contributes to both misdiagnosis and delays in diagnosis by clinicians unfamiliar with the disease. The Gaucher Earlier Diagnosis Consensus (GED-C) Delphi initiative determined which signs and patient co-variables were regarded by experts in GD as most indicative of GD types 1 or 3 in the early stages.

Aims: From the findings of the GED-C expert consensus, to generate a simple web-based 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 Delphi 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 = 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 panel consensus of 100% response rate in each round. Factors identified as major or minor in GD types 1 or 3 are given in the Table. There was 100% agreement that splenomegaly (≥3-fold enlargement) and disturbed occludomotor function (slow horizontal saccades with unimpaired vision) are major signs in GD, and these were assigned a score of 3 in the prototype PSS; other major signs and co-variables were assigned a score of 2. The panel was divided about 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: A prototype PSS to inform GD diagnostic testing has been developed from the GED-C Delphi initiative. The 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 2011-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 cities 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±0.81 respectively. According to clinical history of patients prior to diagnosis, 29 patients (20.7%) were diagnosed due to hemolysis after favism and prolonged neonatal jaundice respectively. 6 patients (4.3%) were diagnosed of G6PD deficiency by screening the patients who applied for soldier recruitment. 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 the 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 seem to be over the Turkey average (2%). Nearly half of the patients had hemolytic anemia 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 RBC antigens. Most common type is warm AIHA which can be either idioptic 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 at 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 danasin. All of the patients who underwent 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 30 patients who were treated with standard dose of Rituximab; two achieved CR and one did not achieve any response. One of two rituximab-responded 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 reorganized by the Clinicas de Referencia Nacional y Grupos de Expertos en Enfermedades Lisosomales (National Reference Clinics and Expert Groups on Lysosomal Diseases [EGLDs]), which created the Guías de Práctica Clínica (Clinical Practice Guidelines) for GD.

Aims: To evaluate the results obtained for 39 patients diagnosed with type 1 GD (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 at 60 UI/kg every 14 days. The enzymatic activity of the β-glucocerebrosidase and the chitotriosidase was determined. We determine 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 hepatomegaly, splenomegaly, and skeletal pain. Additionally, increases in the hemoglobin and platelet 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.
Aims: ABP 798 was compared with rituximab sourced from the European Union (EU). Quality attributes assessed included binding properties (CD20, C1q, FcRn, and Fcγ 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 FcγRIIa, FcγRIIb, and FcγRIIIa (158V) were evaluated in AlphaLISAb competitive binding assays. 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 (FcγRIIa[158V]), used as effector cells. CDC activity was evaluated in a functional cell-based assay using a CD20 expressing human B-lymphoblastoid WIL2-S 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 1.

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<tr>
<th>Assay</th>
<th>ABP 798</th>
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<tr>
<td>CD20</td>
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<td>C1q</td>
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<td>FcγRIIb (158V)</td>
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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.

**PB1850**

**DELAYED EFFECT OF G-CSF ON THE CYTOKINE SECRETION THROUGH G-CSF MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS IN CHILDREN WITH CEREBRAL PALSY**

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Background: Granulocyte colony-stimulating factor (G-CSF) has been widely used to mobilize peripheral blood stem cells. In addition, it has also been tried to reveal the regenerative potential in various neurodegenerative diseases.

Aims: We investigated the short-term and delayed effects of infused G-CSF for peripheral blood stem cell (PBSC) mobilization on the various cytokine secretions in children with cerebral palsy (CP).

Methods: G-CSF (10µg/kg/dose) was administered subcutaneously for 4 days to the children with CP. In first group, blood levels of G-CSF, interleukin (IL)-6, IL-10, insulin-like growth factor (IGF-1), vascular endothelial growth factor (VEGF), and brain derived neurerophic factor (BDNF) as well as mobilized total nucleated cell (TNC)/CD34+ cell counts in peripheral blood were compared between just before G-CSF injection (D+0) and 1 day after 4 days of G-CSF injections (D+5). In second group, cytokine levels were compared between D+0 and 1 month after 4 days of G-CSF injection (D+30). Cytokine levels were measured by enzyme-linked immunosorbent assay.

Results: Baseline levels of G-CSF were significantly increased (p=0.000) and IGF-1 decreased (p=0.011) at D+5 after 4 days of G-CSF administration compared to control group. In contrast, other cytokine levels including IL-6, IL-10, VEGF, and BDNF did not show any significant changes between before and after G-CSF administration. CD34+ cell counts (p=0.000) as well as TNC counts (p=0.000) were significantly increased from D+0 to D+5 in children who received G-CSF compared to children received placebo. Regarding delayed effect of G-CSF administration, G-CSF levels were significantly increased from baseline to D+30 (p=0.000), along with the increase the IL-10 (p=0.035) and VEGF levels (p=0.011) and the decrease of IGF-1 levels (p=0.014).

Summary/Conclusions: G-CSF which administered to mobilize PBSCs could induce the delayed effects on the levels of G-CSF itself as well as other cytokines which could affect on the neuroregenerative potential. Further studies would be warranted to reveal the mechanism and clinical significances of these delayed effect of G-CSF or mobilized PBSCs.
Hematopoiesis, stem cells and microenvironment

PB1852

PD-1 IS HIGHLY EXpressed on memory T-cell subsets residing in bone marrow but not in pE-rhPeRiPhErAl 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 two main T cell subsets: central memory (Tcm) and terminal memory (Ttm), 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 [Bousois VA 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 PD1 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 hematolgy 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-FITC, PD1-APC antibodies on BM cells and 7-AAD was used for to discriminate dead cells during flow cytometry.

Results: PD1 expression by T memory cell subsets is shown in the Table 1 (median with interquartile range). The percentage of PD1+ cells within Tcm CD8+ subset was 34.2%,8.0%33% in BM versus 10.4%,1.5%23% in PB. Similar trend was observed in Ttm subpopulation. Tcm, Tem, Ttm, Tte. Median of Tddn-CD8+ cells were 3.8%±1.05%,22,7%±3.9%,42,2%±7.86%,21,9%±4.07% and 2.6%±0.41%, 6.6%±2.59%, 12.7%±1.25%, 8.9%±0.825% in BM and in PB respectively.

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 naive T cells that in its turn leads to restraining 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 aberrations and are capable to gain them in a culture

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Background: Stromal microenvironment_posesses 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 monolayer and tumor cells suspension 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 RQ-PCR method. BMSC were assessed 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 not detected in patient’s leukemic cells. We also examined BMSC from leukemia patients bearing recurrent genetic abnormalities and in one case the leukemia-specific marker was detected by RQ-PCR - we observed expression of ETv6-RUNX1 gene (0,02%) in BMSC by patient with (12;21) acute lymphoblastic leukemia. At the moment of BMSC culture initiation ETv6-RUNX1 expression in patient’s bone marrow was detected at high level (ETV6-RUNX1/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 the BCR-ABL gene expression was measured during microscopy. Besides BCR-ABL gene expression in BMSC was detected by RQ-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 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 PROGENITORS 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 anti-coagulation 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, underlying poor vascular regulation.

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: Cephalic blood 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+CD45negdimCD133+, CD34+CD45negdimVEGFR2+ and CD34+CD45negdimCD133+VEGFR2+ of CEPCs. The BMI of the patients was calculated and the patients were stratified, those under or equal of 1 year, 1 to 3 years, and equal and over 3 years. The statistical analysis was conducted using t-test (Holm-Sidak) and chi-square (Pearson). Weight/obesity over 85thpercentile. The systolic blood pressure (BP) was measured during the time of follow up.

Results: As a whole, patients who received chemotherapy had increased incidence of BMI>85th percentile. The BMI was never detected in patient’s leukemic cells.
Results: The mean values of CEPCs subpopulation CD34+CD45negdimVEGFR2+ estimated in ALL, ST and Controls were 0.00380(SE=0.00072), 0.00461 (SE=0.00146) and 0.002953 (SE=0.00004) respectively. The mean percentage of CD34+CD45negdimCD133+VEGFR2+ between the ST % B-cell precursors and the mean age of the subjects was 128±80 (mean±2SD) months. The mean hematocrit value increased with age (38.68±2.51% in <5 yrs age group to 0.03031(SE=0.00007), 0.00499 (SE=0.00113) and 0.002663 (SE=0.00037). The correlation of hematocrit value increased with age (38.68±2.51% in <5 yrs age group to 0.03031(SE=0.00007), 0.00499 (SE=0.00113) and 0.002663 (SE=0.00037). The correlation of hematocrit value increased with age (38.68±2.51% in <5 yrs age group to 0.03031(SE=0.00007), 0.00499 (SE=0.00113) and 0.002663 (SE=0.00037). 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 0.000241 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 hypoxic 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 in 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 populations as well as native highlanders from other HA regions. We also assessed the age variation of BM B-cell precursors varied during 1-3years 0.0031(SE=0.00066) and >3 years 0.00343(SEC=0.00081). The levels of CD34+CD45negdimCD133+VEGFR2+ between the 1st year after chemotherapy was 0.0092 (SE=0.0037), 1-3 years 0.0034(SE=0.00063) and >3 years 0.0033(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 0.000241 to 0.01525).

Methods: 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.

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.

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 cytophenias 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. Statistical analysis: multiple regression to analyse the dependence of BCPs from the variables age, gender, obesity, hypertension, diabetes and cholesterol level. In all other datasets, the correlation of 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 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.

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>% of total BCPs</th>
<th>BCP/total cells</th>
<th>BCP/CD34+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 years</td>
<td>2.8% (0.35-3.8)</td>
<td>0.00499 (SE=0.0037)</td>
<td></td>
</tr>
<tr>
<td>7-18 years</td>
<td>4.3% (3.2-5.3)</td>
<td>0.00613 (SE=0.0037)</td>
<td></td>
</tr>
<tr>
<td>19-35 years</td>
<td>6.1% (4.9-7.3)</td>
<td>0.00726 (SE=0.0037)</td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: In a normal population BM B-cell precursors varied mainly with age, but were also dependent on technical peculiarities of operators and equipment. Analysis by phenotype and as percentage of total cells was more accurate and less susceptible to variation.

PB1857

PERISTIN/BIGH3 RATIO AS A PROGNOSTIC MARKER OF IDIOPATHIC THROMBOCYTOPENIA AFTER ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION FOR THE PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

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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-β 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 BIG3H.

Methods: We reviewed twenty patients who transplanted with matched sibling donor for acute myelogenous leukemia at Kyungpook National University Hos- pital 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; (+5), 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 (40%) in CR2. Thirteen patients (65%) received myeloablative conditioning regimen. The median dose of CD34+ cell was 3.67×10^6/kg (range, 1.5-7.67×10^6/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 BIG3H or Periostin (p=0.128). However, BM with thrombocyto- penia manifested the low periostin/BIG3H 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/BIG3H might represent the status of BM niche during the homeostasis and regeneration of hematopoiesis. High periostin/BIG3H ratio could predict the recovery of the idiopathic thrombocyto- penia.

PB1859

LABEL-FREE IMAGING BY AUTO-FLUORESCENCE PERMITS IDENTIFICATION OF ERYTHROID PRECURSORS IN BONE MARROW AND DETECTS CHANGES OF SOLUBILITY OF HEMOGLOBIN IN ERYTHROCYTES

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Background: In the fluorescence lifetime imaging (FLIM) technique, the image contrast is created by determining the delay of the fluorescence photon emis- sion at each pixel of the image and transforming it in pseudo-colors. This delay, also called lifetime depends on the type of molecules and their physicochemical characteristics.

Aims: We investigated the utility of this technique for the characterization of erythropoietic cell line and changes in the solubility of hemoglobin.

Methods: We used unstained BM smears of 24 normal BM and 8 megaloblastic anemia patients and unstained peripheral blood smears of 10 patients with sickle cell disease. Images were captured with a confocal microscope (a HPM-100-40-Hybrid detector and excitation at 405 nm (diode laser,80 MHz). In order to create equivalent images of the cytological smears, pseudo-colors were attributed to different lifetime ranges. Images were compared with May-Grünwald-Giemsa (MGG) stained smears.

Results: FLIM created highly contrasted images, where different cell types could be easily recognized by their similarity with MGG images. Erythrocytes exhibited the shortest lifetimes (210.42±2.1 ps). Normal shaped erythrocytes in smears of sickle cell patients showed similar values (214.63±3.1 ps), whereas crenated erythrocytes as well as drepanocytes revealed significantly elevated values (314.26±6.7 ps and 312.56±6.0 ps respectively). Regarding erythro- poiesis, the cytoplasm of erythroblasts showed significantly shorter lifetimes (623.52±27.1 ps) than that of myeloblasts (835.92±198.4 ps) and the same was the case when comparing the nuclei (erythroblasts: 895.42±262.8 versus myeloblasts: 1166.42±287.9 ps). The same differences could be found in megaloblastic anaemias. There were no significant differences between the FLIM val- ues 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 identifica- tion. It allowed also to distinguish between erythroid and myeloid precursors cells and indicates the major physico-chemical changes during the process of falcination.

PB1858

ASSOCIATION WITH OMENN SYNDROME AND CYSTINURIA: CASE REPORT

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Background: Omenn syndrome is one type of combined immunodeficiency, characterized with hepatoplenomegaly, lymphadenopathy, recurrent infections and has an autosomal recessive pattern of inheritance. T lymphocyte count can be normal in peripheral blood but their functions are impaired. B lymphocyte and has an autosomal recessive pattern of inheritance. T lymphocyte count 1092/mm3, B lymphocyte count 0.02×10^6/mm3. NK count 332/mm3 were found respectively. Blood sample of patient was sent to Erasmus for genetic analysis. The patient had no full-match family donor. Hence, hap- loidentical bone marrow transplantation from her father was planned. In prepara- tion for bone marrow transplantation, bilateral kidney stones were showed in abdominal CT. Cystinuria was detected in urine and thought to be bilateral cys- teine Stone. Percutaneous nephrolithotomy operation was performed, then the patient was given scholl solution. Stone analysis revealed to be cystine stone.

Results: Association with two different diseases inherited autosomal recessive is very interesting. Challenging incident that can be caused by a reason or it can be only coincidence. In Omenn Syndrome is known to be sequencing alteration of cysteine and tyrosine amino acids. Perhaps, cystine stones took form as a result of this alteration.

Figure 1.

Summary/Conclusions: The periostin/BIG3H might represent the status of BM niche during the homeostasis and regeneration of hematopoiesis. High periostin/BIG3H ratio could predict the recovery of the idiopathic thrombocyto- penia.

PB1860

TWO HEMATOLOGICAL MALIGNANCIES, SIMULTANEOUS OR CONSECUTIVE OCCURRENCE. EXPERIENCE OF A CENTER

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Background: Numerous reports of coexistence or consecutive occurrence of hematological malignancies are found in the literature.

Aims: this study reports cases of patients with two hematological malignan- cies treated in a single center.

Methods: Retrospective study of patients with two malignancies occurring simultaneously or consecutively in patients in a hematology department during a 15 years period.

Results: Thirteen (13) cases were identified (5 women, 8 men). Their demo- graphic characteristics, diagnoses, treatment and overall survival are shown...
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>Disease 1</th>
<th>Disease 2</th>
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<tr>
<td>CLL</td>
<td>MDS</td>
</tr>
<tr>
<td>CML</td>
<td>PV</td>
</tr>
<tr>
<td>DLBCL</td>
<td>ET</td>
</tr>
<tr>
<td>CMML</td>
<td>FL</td>
</tr>
<tr>
<td>HL</td>
<td>SMZL</td>
</tr>
<tr>
<td>HCL</td>
<td>MGUS</td>
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</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.

Hodgkin lymphoma - Clinical

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 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 hazard 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).

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.
PB1862
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 77.6% 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 63.8% vs 73.2%, (log rank; p=0.066) and lymphopenia (5-year EFS 50.0% vs 66.7%, kog rank p=0.025), while age, gender, B symptoms, ESR>50 mm/h, anaemia 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 identified 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 A and B were considered as one group of patients and the negative prognostic factors were merged. Finally, we developed prognostic model for identifying patients at low (0 factors), intermediate (1 factor) and high risk (2-3 factors) for poor outcome (p=0.000). According to this model, in the examined group 34 (22.8%) patients had low, 64 (43.0%) intermediate and 51 (34.2%) high risk for poor outcome, with 5-years OS of 100%, 84.6% and 60.8%, respectively.

Summary/Conclusions: According to the score which we developed, ABVD is very effective in the subgroup of advanced HL patients without large tumor mass and without identified risk factors.

PB1863
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 for advanced Hodgkin’s disease (HD) and non-Hodgkin’s lymphoma (NHL). Aims: we report here our experiment of escalation in case of positive PET after 2 cycles of ABVD (PET 2) in patients with advanced Hodgkin lymphoma. Methods: Among the 102 patients with Hodgkin lymphoma treated between 2008 and 2016, 50 patients had advanced disease (Stage III or IV of Ann Arbor). The majority of patients were treated on front line by ABVD (47 patients), 2 by BEACOPP and 1 by VABEM. All patients underwent PET evaluation at diagnosis and after 2 cycles of treatment. The analysis of the metabolic response was carried out according to the Deauville criteria.

Results: The median age of the patients was 48 years (min-max: 19-85). 20 patients (40%) had an unfavorable prognosis, 24 (48%) had an intermediate prognosis. 11 patients (22%) were refractory to the ABVD protocol and had an escalation of treatment. The median PFS was 66 months (47-85). The median overall survival was not achieved; OS at 60 months was 65%. We found no difference in survival between patients with negative PET and those with positive PET in case of escalation of treatment. The study of PET 2 response, its impact on survival, as well as escalation of treatment will be presented to the EHA with update of follow-up.

Summary/Conclusions: This study evaluated the value of escalating treatment in patients with advanced PET 2 in patients with advanced Hodgkin lymphoma treated in first-line by ABVD. This management aims to reduce the toxicity of 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.

PB1864
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 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. 18F-FDG PET/CT was used to assess response in HD and NHL pts after standard chemotherapy by using standardized uptake values (SUV) for each lesion. SUV was compared with the SUV of the same lesions pre-treatment and SUV of other lesions of the same patient.

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 pts, 50 (90.1%) HD pts, 85 (60.8%) NHL pts and 47 (87.0%) 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. These pts had one hypermetabolic lesions and were considered for radiotherapy, while pts with more than one nodal or extranodal lesions after completion of standard chemotherapy were considered for high dose chemotherapysautologous stem cell transplantation (ASCT).

Summary/Conclusions: 18F-FDG PET/CT 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.
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 minimum 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. The 3-year event-free survival (EFS) in bcl-2+ patients had lower RR 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 RS was an independent factor of poor prognosis. 3 years 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 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, Vincristine 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). The observed RR response rate (CR) was 55.7% [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 a more powerful factor of poor prognosis than bcl-2+ cells.

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.
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.

The red blood cell distribution width (RDW) is associated with short- and long-term outcomes of various malignancies. The prognostic value of the RDW in cHL remains unknown.

Aims: The aim of this study was to analyze the prognostic significance of RDW in cHL patients.

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 were 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). Multivariable Cox regression analysis showed that RDW > 14.5% was an independent prognostic factor for EFS (hazard ratio [HR] 3.801, 95% confidence interval [CI] 1.41-14.45, 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 as 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 clinical-diagnostic pathological 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: Objectives of this study were 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

Indolent Non-Hodgkin lymphoma - Clinical
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=30 [62.3%]) with 95% CI: 54.2–70.2). Patients achieving CR (confirmed, n=20 [41.2%]; unconfirmed, n=15 [18.1%]) and 23 (27.7%) 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 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 not related to lymphoma or occurred during remission.

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]).

PB1872
A PROSPECTIVE PHASE 2 TRIAL EVALUATING MONOTHERAPY WITH OFATUMUMAB FOR RELAPSED/REFRACTORY SPLENIC B-CELL MARGINAL ZONE LYMPHOMA (MZO) - SAFETY ANALYSIS RESULTS
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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 6 years, range 49 86 years) with 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 used in hematological practice (comorbidity index (CCI; ≤3∕>3), ECOG performance status (PS; <2/≥2) and 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 incor- porated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Results: For 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 to indentify functional, cognitive, social, nutritional and psychological parameters of the general health status.

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.

PB1873
INDOLENT B-CELL LYMPHOPROLIFERATIVE NEOPLASMS – A SINGLE CENTRE EXPERIENCE
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Background: 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 6 years, range 49 86 years) with 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 used in hematological practice (comorbidity index (CCI; ≤3∕>3), ECOG performance status (PS; <2/≥2) and 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 incor- porated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Results: For 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 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 incor- porated 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.
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 AEs 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 GGT increase (grade 3, at baseline grade 2), 9 cases of ALP increase (all grade 1-2), 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 1: List of AEs.**

<table>
<thead>
<tr>
<th>Drug-related AEs</th>
<th>N of events (any grade 1-2)/n</th>
<th>Non-drug related AEs</th>
<th>N of events (any grade 1-2)/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>5/3</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2/2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>1/1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>4/4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Dizziness</td>
<td>2/2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Bone pain</td>
<td>1/1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2/2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1/1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Rash</td>
<td>2/2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Alopecia</td>
<td>1/1</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

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.

**PB1875**

**TREATMENT PATTERNS AND TREATMENT RESPONSE IN PATIENTS WITH FOLLICULAR LYMPHOMA IN ROUTINE CLINICAL CARE – A UNITED STATES ELECTRONIC MEDICAL RECORD DATABASE STUDY**

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1Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, 2Xcenda LLC, Palm Harbor, United States

**Background:** FL represents 70% of all indolent non-Hodgkin lymphomas, and it is widely recognized that FL is a heterogeneous disease, with patients presenting with differing amounts of tumor burden and prognostic indicators. The NCCN guideline recommends using rituximab as a single agent or in combination with other chemotherapies as first-line therapy (1LT) or second-line therapy (2LT). No recommendations are provided beyond 2LT.

**Aims:** To evaluate treatment patterns and associated response in patients with newly diagnosed FL in routine care in the US.

**Methods:** Newly diagnosed FL patients aged ≥18 years were selected from Humedica, a large, national US EMR database between 01/01/08 and 07/31/15 if they had ≥1 visit recorded in the EMR with FL diagnosis codes. The date of the first FL record was the index date. Patients were followed from index until end of continuous activity, progression to diffuse large B-cell lymphoma (DLBCL), death, or end of study period (09/30/15) and were evaluated for FL treatment patterns and treatment response. Possible remission was defined as no additional chemotherapy and no supportive care use or receipt of supportive care <30 days after end of line of therapy (LOT) for <30 days. Lack of remission was defined as receipt of supportive care >30 days after end of LOT for >30 days.

**Results:** Of the 3,756 patients selected into the study, 1,346 (35.8%) initiated 1LT, and median (interquartile range [IQR]) time to therapy was 1.3 (0.5–5.9) months. Overall, treatment regimens were mainly rituximab-based. In 1LT, more patients initiated combination chemotherapy (61.4%) vs single-agent chemotherapy (38.6%). Bendamustine+rituximab (26.9%) and R-CHOP (15.1%) were the most common combination regimens, and rituximab (33.1%) was the most common single agent. Median (IQR) duration of 1LT was 4.3 (1.7–10.4) months. At the end of 1LT, 54.7% (n=736) had evidence of remission, 25.5% (n=344) progressed, and 1.6% (n=22) had no evidence of remission.

**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.

**PB1876**

**PET-CT AND BONE MARROW BIOPSY IN STAGING FOLLICULAR LYMPHOMA IN A SINGLE INSTITUTION**

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**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 show bone marrow involvement. The false positive rate is one factor considered in the FLIP-I and FLIP-II prognostic index. Position 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 (>90-100%) 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 since June 2005 to October 2016. Of them, 64 who underwent both BMB and PET-CT before treatment were evaluated. The BMB was evaluated by hematopathologist and the interpretation of PET-CT images was interpreted by a nuclear radiologist. Positive BMB was defined as the presence of CD20 + CD10 + 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 more than those in liver or mediastinum.

**Results:** Thirty-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 sensitivity 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.**

<table>
<thead>
<tr>
<th></th>
<th>BMB –</th>
<th>BMB +</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET -</td>
<td>27(87%)</td>
<td>20(61%)</td>
<td>47(73%)</td>
</tr>
<tr>
<td>PET +</td>
<td>4(13%)</td>
<td>13(39%)</td>
<td>17(27%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>31(100%)</td>
<td>33(100%)</td>
<td>64(100%)</td>
</tr>
</tbody>
</table>

**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-
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 (1LT).1 Recommended therapies for 1LT 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 used to identify newly diagnosed FL patients from Humedica, a large US EMR data base, between 01/01/08 and 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 the 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 (85.1%). For combination therapy, bendamustine+R (43.8%) and R-CHOP (24.6%) 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.

Reference

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 PUGLIESE G. Tarantini1,2, S. Capablo1, F. Angrilì3, N. Cassanvalli4, M. Ciminelle5, C. Bilinti6, G. Milazzo7, A. Guarini6, P. Mazza6, N. Di Renzo10, V. Pavone11
1Dipartimento Oncologia ematologica ASL BT, U.O.C. di Ematologia con Trapianto ASL/BT Barletta - Italy, Barletta, 2Ematologia, Foggia, 3Ematologia, Pescara, 4Ematologia, Sa Giovanni Rotondo, 5Ematologia, Potenza, 6Ematologia, Reggio Calabria, 7Università di Bari, Cattedra di Ematologia, 8RRCs Oncologico Bari, Ematologia, Bari, 9Ematologia, Taranto, 10Ematologia, Lecce, 11Ematologia, Tricase, Italy

Background: Results from phase 3 "Stil" and "BRIGHT" trials demonstrated the effectiveness of the combination Bendamustine-Rituximab (BR) compared to R alone as frontline treatment for advanced Follicular Lymphoma (FL), emphasizing BR as a standard strategy in this subset of patients. However, only a few studies investigated the efficacy and safety of R maintenance after frontline BR, indicating a significant beneficial effect of R administration in term of PFS but not OS, in keeping with a good toxicity profile even over two years of treatment.

Aims: In this study, we evaluated the role of maintenance therapy with R after induction with BR in previously untreated FL, and compared its efficacy and safety profile with recent publicly available results of "Stil" trial.

Methods: FL patients were treated with a maximum of 6 cycles of B-R (Bendamustine 90 mg/m² 8days 1+2), Rituximab 375 mg/m² every 28 days followed by 2 additional cycles of Rituximab monthly. Patients showing complete response [CR] or partial response [PR] were then allowed to receive R maintenance [375 mg/m²] administered every two months. To date 118 pts (65 women and 53 men) with FL have been recorded. Median age was 61 years (range 28-86); 15 (13%), 41 (35%), 62 (52%) pts had respectively stage II, III and IV; median number of nodal areas was 4, bone marrow involvement was found in 56 (47%) pts, and median FLIPI was 3.

Results: Among the 118 pts, 94 were evaluable for response and safety. The overall response rate [ORR] was 89.2% with 83 pts achieving a remission after BR therapy. The CR rate was 84.4%, 7 pts had partial response, 5 pts (6.1%) had stable disease, whereas 3 (3.5%) showed no response to BR and had a progressive fatal disease. All of the pts achieving remission received the full planned 2 years Rituximab maintenance treatment and, among them, 24 pts (28.9%) were administered with R over the first two years. Primary adverse events recorded were of grade 3 ad 4 in 25% of cases. Infectious (grade 3-4) and neutropenia (grade 3) were the most common adverse event, no additional unexpected toxicities were observed, whereas no occurrence of secondary malignancy was registered so far.

Summary/Conclusions: Our data, compared with recent reports about the role of Rituximab maintenance, support the efficacy of BR as backbone treatment of choice in previously untreated advanced FL. These results, moreover, are in line with those of other studies indicating that Rituximab standard maintenance and also over 2 years for FL appears safe and well tolerated, with no additional toxicities.

PB1878
ROLE OF F-18 FDG-PET/CT IN DETECTING LYMPHOMATOUS BONE MARROW INVOLVEMENT IN THE INITIAL STAGING OF PATIENTS WITH Hodgkin LYMPHOMA B. Hernández Ruiz1,*, C. Calle Primo1, D. Buenasmañanas Cervantes1, A. Mayoralas Tendero1, R. Vanegas Uribe1, M. Nebro Luque1, A. Garcia Vicente2
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Background: The role of F-18 FDG-PET/CT for the assessment of bone marrow involvement 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 sensitivity, specificity 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, median age 32 years), 48 FL (23 male, 25 female, median age 55 years). 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 these patients. PET/CT was positive in bone marrow biopsy (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 a 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 BMBPET+. 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 another of orbital involvement by contiguity. Fourteen patients of 48 patients with HL had BMB+. Of these 14 patients with bone marrow involvement by biopsy, 5 patients had BMBPET- 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).

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>BMB+</th>
<th>BMB-</th>
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<tbody>
<tr>
<td>HL</td>
<td>PET-</td>
<td>PET+</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>DLBCL</td>
<td>PET-</td>
<td>PET+</td>
</tr>
<tr>
<td>48</td>
<td>9</td>
<td>5</td>
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</table>

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 hystological 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.

PB1879
Abstract withdrawn.
PB1880

PREDICTIVE FACTORS FOR INFECTIONOUS ADVERSE EVENTS IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA TREATED WITH BENZAMUSTINE-RITUXIMAB (R)R) MAINTENANCE. RESULTS OF A RETROSPECTIVE STUDY

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Background: The combination of benzamustine (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 chemotherapy regimens.

Aims: We performed a retrospective analysis at our institution in patients treated with BR 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 grading scale. We compared the patients with or without infection episodes during the observation period and found out what 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, and 37 (58%) 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, 25% as second line and above. Benzamustine was administered either at the dosage of 100 or 90 mg/m2 iv 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 2-6), 13 patients (20%) 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-sulfametoxazole against Pneumocystis jiroveci pneumonia was given to all patients. Twenty two patients (34%) had at least one infection. Bacterial pneumonia was identified in 22 patients, varicella zoster virus infection in 4/22, cytomegalovirus retinitic fever (NF), lymphopenia, infections episodes and the occurrence of second tumors - were recorded according to the CTCAE v4.0 grading scale. We compared the patients with or without infection episodes during the observation period and found out what did not. Univariate analysis with Fisher’s exact test was used to evaluate the potential risk factors.

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 neoplasms 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

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Background: Indolent Non Hodgkin Lymphomas (INHL) are slow growing lymphomas that usually arise 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 subtype, site of involvement and CT and PET/CT scan were studied. Extrapolated 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: A total of 265 patients diagnosed with INHL. 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 59.26 (12.39). Among these patients, 45 patients (51.1%) had small lymphocytic lymphoma (SLL), 38 patients (45.3%) had chronic lymphocytic leukemia (CLL), 20 patients (22.7%) had follicular lymphoma (FL), 15 patients (17%) had marginal zone lymphoma (MZL), 6 patients (8%) had mantle cell lymphoma (MCL) and 2 patients (2%) 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 associations between patients with INHL subtype and extra nodal sites involvement. (P-value=0.001). 60% of patients with MZL, 50% of patients with MCL, 20% of patients with FL and 8.9% of patients with CLL had extranodal sites involvement. 11 patients (12.5%) from all INHL had transformed to DLBL. There was significant association between INHL subtypes and transformation to aggressive NHL. (P-value=0.002). 7 from 20 patients with FL (35%) and 4 from 45 patients with CLL (8.9%) had transformed to DLBL. Mean LDH level (886.1 U/L) in patients with transformation to DLBL was significantly higher than mean LDH level (490.7 U/L) in other patients. (P-value=0.0004). There was no significant association between mean age and mean albumin level with risk of transformation to DLBL. The overall survival rate was 56.8%. 10 years and 5 years survival rates were 47% and 60% respectively. Mean survival time in patients with MCL (31.8 months) was significantly lower than mean survival time in patients with follicular (85.48 months), MZL (90.6 months) and CLL (103.6 months) patients . (P-value=0.0004). There was no significant difference in survival between patients who transformed and patients who didn’t transform to DLBL.

Summary/Conclusions: Prevalence of INHL among patients with NHL in North Jordan is 33.2%. The most common INHL subtypes in our patients were...
Cll (51.1%) and FL (20.7%). These findings are significantly different from Saudi Arabia and Western Countries in which FL is the most common subtype. FL and Cll are associated with higher risk of transformation to DLBCL. High LDH level is considered a risk factor for transformation to DLBCL in our patients. MCI is associated with significantly lower mean survival time than other NHL subtypes.

PB1883

OCULAR ADNEXAL LOW GRADE LYMPHOMA TREATMENT OUTCOMES AND LONG TERM FOLLOW UP: A SINGLE CENTRE EXPERIENCE

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Background: Ocular adnexal lymphoma (OAL) accounts for 1-2% of Non-Hodgkin Lymphomas (NHL) and 8% of all extra-nodal sites. The majority of cases, >95%, are of B cell origin and 80% are low grade lymphomas. Secondary ocular involvement occurs in approximately 2.4-5.3% of patients with advanced systemic NHL. Marginal zone lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma is reported in approximately 50% of patients. Current treatment options for low grade OAL include radiotherapy and chemotherapy. Chlamydia Psittaci DNA has been reported in up to 80% of tumor biopsies from patients with OAL suggesting a possible value of anti-Chlamydia Psittaci antibiotic therapy.

Aims: To report a single centre’s experience in the outcomes of patients diagnosed with OAL over a 13 year period.

Methods: A Retrospective cohort of patients with low grade OAL treated in a single Centre between 2003 and 2016 was analysed. 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 treated with full line chemotherapy, single agent Chlorambucil in 78% (7/9) and 2 patients received Fludarabine based chemotherapy. 30% (6/20) received first line radiotherapy, 24Gy in 12 fractions in 67% (4/6), and 25% (5/20) 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).

Table 1. Summary of the management modalities of ocular adnexal low grade non-Hodgkin lymphoma.

<table>
<thead>
<tr>
<th>Modality</th>
<th>Number of Patients</th>
<th>Median Age (Range)</th>
<th>Follow Up Time</th>
<th>Disease Control</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiotherapy</td>
<td>12</td>
<td>61.5 years (45-85)</td>
<td>9.5 years (1-14)</td>
<td>80% (16/20)</td>
<td>2/6 (3/5 local recurrence, 2/5 extra-ocular)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>8</td>
<td>61.5 years (45-85)</td>
<td>9.5 years (1-14)</td>
<td>55% (5/9)</td>
<td>3/6 (1 relapse, 2 relapses)</td>
</tr>
</tbody>
</table>

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 localized OAL. The exact role of radiotherapy 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 (PCFCL). Primary Cutaneous Diffuse Large B-Cell Lymphoma (PCDCLBCL) is an aggressive subtype with a fatality rate of 50%. The Cutaneous Lymphoma International Prognostic Index (CLIPi) can stratify indolent subtypes, but criteria do not include age. Here we present our single centre analysis of clinicopathological features and outcomes of patients with PCBCL.

Aims: To analyze clinical and laboratory characteristics such as age, lesion characteristics, hemato-morphological parameters, and treatment modalities in order to determine their impact on progression free survival (PFS) in PCBCL.

Methods: This is a retrospective study of patients of selected patients treated at Moffitt Cancer Center between January 1990 and December 2016. Patients were identified using our PCBCL database and diagnosis was verified by independent hematopathologists and dermatopathologists. Staging was determined according to ISCL/EORTC recommendations. Demographics, lymphoma subtype, stage, disease course, and CLIPi scores were collected. Kaplan Wallis ANOVA and Fisher’s Exact tests were used to compare differences among the four subtypes for continuous and categorical variables, respectively. Kaplan Meier curves were produced to estimate PFS for different strata, and differences among the strata were tested using the log-rank test.

Results: We identified 37 patients who met diagnostic criteria for PCBCL (35% PFCL, 40.5% PCMZL, 13.5% PCDCLBCL, and 11% indolent, unspecified). Male/female ratio was 2.4:1. 51% of patients were ≥60 years old (yo) and 49% were <60 yo. 94% had stage T1 disease, 27% T2, and 19% T3. Median PFS for patients <60 was 1.1 years, but was not reached for those ≥60. Mean follow-up time was 2.6 years for all patients. Log rank test showed a statistically significant difference in PFS between the two age groups (p<0.01). This was consistent when comparing PFS by age in both high (PCDCLBCL) and low grade (indolent) subtypes. PFS according to stage in indolent subtypes showed a marginally statistically significant difference (p<0.06). Stratification of patients according to CLIPi did not show a significant difference in PFS among indolent subtypes.

Summary/Conclusions: We found that age is a highly statistically significant prognostic parameter in PCBCL, as patients ≥60 years old had a longer PFS compared to younger patients, even after adjusting for stage and CLIPi. This is an interesting finding as most NHL studies demonstrated a negative impact of advanced age on PFS. Our results suggested that age is a possible novel prognostic indicator in patients with PCBCL, however validation on a larger sample set is needed.

PB1885

EPIEDEMIOLGY, CHARACTERIZATION AND THERAPEUTIC MANAGEMENT OF MARGINAL ZONE LYMPHOMA: A SINGLE-CENTER EXPERIENCE

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Background: Marginal zone lymphomas are a group of relatively uncommon lymphomas whose cells are derived from B lymphocytes of the ”marginal zone” of the secondary lymphoid follicles.

Aims: The objective of this study is to review our series evaluating the epidemiology, clinical presentation, morphological, immunohistochemical and molecular characterization and therapeutic management in a tertiary hospital.

Methods: We evaluated a total of 56 patients diagnosed between May 2008 and February 2017. We collected the epidemiological and clinical data, including location, clinical stage, FLIPI and associated risk, antigenic stimulus, symptomatic localization, histological diagnosis and anticipated side effects. As treatment we reviewed the levels of LDH, beta2microglobulin, ES, peripheral blood (PB) immunophenotype and studied the morphological, immunohistochemical and molecular characteristics (MALT1 translocation and H3F3A mutations).

Results: A total of 37 patients who met diagnostic criteria for PCBCL (35% PFCL, 40.5% PCMZL, 13.5% PCDCLBCL, and 11% indolent, unspecified). Male/female ratio was 2.4:1. 51% of patients were ≥60 years old (yo) and 49% were <60 yo. 94% had stage T1 disease, 27% T2, and 19% T3. Median PFS for patients <60 was 1.1 years, but was not reached for those ≥60. Mean follow-up time was 2.6 years for all patients. Log rank test showed a statistically significant difference in PFS between the two age groups (p<0.01). This was consistent when comparing PFS by age in both high (PCDCLBCL) and low grade (indolent) subtypes. PFS according to stage in indolent subtypes showed a marginally statistically significant difference (p<0.06). Stratification of patients according to CLIPi did not show a significant difference in PFS among indolent subtypes.
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 mantle cell lymphoma (17 patients, 30.4%), followed by MALT: 10 pulmonary (17.9%), 10 gastric (17.9%), 5 cutaneous (8.9%), 5 ORL (8.9%), 2 (3.6%), 1 hepatic, 1 thyroid and 1 lralcim gland (1.8%) and nodal marginal zone lymphoma (3 patients, 5.4%). Five of them presented with multi- focal 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 antigentic 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%). Six of seventeen cases (35.3%) showed light reaarrangements. Antinuclear antibodies were positive in 15 of 32 patients (46.9%). Flow cytometry and the bone marrow aspiration-biopsy. Recently, B-RAFV600E mutation was demonstrated in%100 of Tiiacci HCL case series.

Summary/Conclusions: Marginal zone lymphoma is an indolent lymphoma 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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Background: Hairy cell leukemia(HCL) is a B cell lymphoproliferative disorder, presenting with splenomegaly, hepatomegaly and bone marrow infiltration. HCL accounts for%4,5 of non-Hodgkin lymphomas, more commonly seen in man. Diagnosis is based on the examination of peripheral blood smear, flow cytometry and the bone marrow aspirate biopsy. Recently, B-RAFV600E mutation was demonstrated in%100 of Tiacci HCL case series.

Aims: Aim of our study is to investigate the frequeny of B-RAFV600Emutation and other rare mutations of B-RAF (B-RAFQ464E, B-RAFQ466E, B-RAFQ469E) and their relation with clinical data and treatment responses.

Methods: Charts of 13 patients diagnosed with HCL were analyzed retrospectively. Patients’ clinical parametres 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, Qiaigen) After spec-trophotometric measurement of DNA; common and uncommon mutations of B-RAF were investigated. (Qiagen PyroMark Q24 system, Therascreen BRAF Kit). All patients received maintenance with rituximab after obtaining a complete (CR) or partial remission (PR) in 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 lymphoma 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.

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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Background: Follicular lymphoma (FL) is characterized by a course of relapses and increasingly shorter responses to the consecutive treatments. In first relapse after immunochemotherapy, in patients who are not considered refrac- tor to rituximab, there is no standard treatment. In Spain, bendamustine in association with rituximab (BR) has not been approved for this indication. Nev- ertheless this combination has shown high efficacy and excellent tolerance in patients previously treated with and without rituximab.

Aims: To evaluate the efficacy and safety of the bendamustine-rituximab asso- ciation 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 com- passionate 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 33 in 48%. Bulky mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow involvement was detected in 60%. 88% of patients had received more than one previous treatment, with an average of 1.7 (1-5) and the most frequent was CHOP-R in 66% followed by CVP-R in 11%. All patients had previously received rituximab and only 3 patients (7.3%) could be considered refractory. All patients received BR (B-90 mg / m2 D1-2, R-375mg / m2 D1). Median cycles 5.1 (1-8). Support with G- CSF was used in 27.5% of cycles. Maintenance with rituximab after obtaining a complete (CR) or partial remission (PR) was administered in 42% of patients.

Response: The overall response rate was 95.1% (65.8% CR-ICR / 29.3% PR). With a median follow-up of 25 months (6-92) the median response duration was 41.9 months (32.8-51.1) and the median progression-free survival (PFS) was 57 months (27.4-86.5) with no impact neither by the number of previous treatements (1 vs 2) (P=0.69) nor by the age (<70 vs ≥70) (P=0.9). Patients who received maintenance with rituximab after BR had a significantly longer median PFS than without (NR vs 32) (P=0.004). Toxicity: No treatment-related death was recorded. 42% and 36.6% of the patients presented G3-4 neutrope- nia. One patient died due to febrile neutropenia. One patient received cotrimoxazole prophylaxis and 3 opportunistic infections were recorded (1 P. jirovecii pneumonia in a patient without prophylaxis).

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 radio- therapy in our department.

Methods: 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.

PB1889

USE OF LIPEGFILGRASTIM IN CLINICAL PRACTICE FOR THE PROPHYLAXIS OF CHEMOTHERAPY-INDUCED NEUTROPENIA IN LYMPHOMA PATIENTS: INTERIM RESULTS OF A PAN-EUROPEAN NON-INTERVENTIONAL STUDY

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Background: Lipegfilgrastim (Lonquex®) 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.

PB1890

TUBERCULOSIS IN ACUTE LEUKEMIA- AN ANALYSIS OF CLINICAL CHARACTERISTICS AND IMPACT ON MANAGEMENT IN 25 PATIENTS

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Background: Patients with acute leukemia represent an immune-compromised population, with innate, humoral and cellular immune pareshes. 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 records 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-tuberculous 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 with appropriate therapy, 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 more serious septic episode, an empirical 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 immunosuppression 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.

![Figure 1](Image)

**Summary/Conclusions:** - Current antimicrobial resistance, especially concerning G- in our study, is particularly worrisome due to development of resistance to all available antimicrobial agents. The incidence of multi-resistant G- is not very high. - Clinical presentation in MRB infections is more serious in our experience, and the mortality doubles in relation to the difficulty to establish appropriate treatment. - Severity sings at infection diagnosis in MRB carriers had led us to a change of empirical antibiotic therapy. - As reported in previous literature, prevention of transmission, a quick establishment of diagnosis and an effective treatment, along with a correct and limited use of antibiotic therapy could decrease the development of MRB.

**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 Leishmania donovani, and one case of Plasmodium falciparum, Parvovirus 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 Leishmania 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 HIV/AIDS and FUO 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 measure the utility of BMT in FUO patients.

Methods: We reviewed retrospectively the bone marrow biopsy results of the inpatients 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 Petersdorf 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 lymphomatous aggregates; one secondary hemophagocytosis (3.2%) and one mastocytites 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 an aseptic reaction was observed in culture and 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 diagnosed. 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 in patients with HIV/AIDS is essential for the pathologic diagnosis in these patients. Bone marrow biopsy is still a useful ancillary procedure for establishing the diagnosis of FUO, only if used in the adequate context.

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 Hospital (CNUNH) 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±10.5, 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 ≥3 organ system dysfunction (OR, 18.5; 95% CI, 4.4-77.0; P<0.001). Hematologic cancer patients had higher mean PRISM III score (16.4±4.4 vs 12.2±8.6; P=0.51) and higher risk of sepsis (39.3% vs 13.0%; P=0.02). These results are comparable to solid cancer 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 multdrug-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 median 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 hypotetical 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 6.3%, the clinical effective was 13.1% (p<0.01). 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.

PB1896
BONE MARROW CYTLOGICAL CHARACTERIZATION OF PATIENTS WITH HIPERREACTIVE MALARIAL SPLENOMEGALY
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Background: Hyperreactive malarial splenomegaly (HMS) is a common cause of massive splenomegaly in malarial-endemic areas. At present, diagnosis of patients with suspected HMS in tropical medicine departments of European hospitals is relatively frequent due to immigration and the return of missionaries and NGO workers after long periods in tropical countries.

Diagnostic protocols for HMS usually include a cytological study of bone marrow (PB) 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: The aim is to define the bone marrow cytological pattern of patients with confirmed HMS, as well as of HMS patients with associated viral (HIV, HBV, HCV) or parasitic diseases.
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 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 lymphocytosis 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+IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid/erythroid ratio</td>
<td>3.1±1.8</td>
<td>2.8±1.7</td>
<td>3.4±1.6</td>
<td>2.5±1.0</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>5±</td>
<td>12±5</td>
<td>7±6</td>
<td>6±5</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>31±5</td>
<td>5±6</td>
<td>27±5</td>
<td>25±6</td>
</tr>
<tr>
<td>Plasma cells (%)</td>
<td>54±</td>
<td>8±2</td>
<td>8±1</td>
<td>6±1</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.

Figure 1.

Summary/Conclusions: As far as we know, this is the largest series of HMS bone marrow analyzed. Identification of common cytological findings in all the groups studied allows defining a characteristic cytological pattern for HMS. The reason for these findings could be related to an aberrant chronic immune response caused by a continuous exposure to malaria parasites. Only bone marrows of HIV infected patients present additional specific alterations (decreased cellularity and high proportion of atypical lymphocytes). Some authors hypothesize that HMS could eventually evolve to chronic lymphocytic leukemia, hairy cell leukemia or splenic lymphoma with villous lymphocytes, so a special follow-up would be advisable for those patients with a high proportion of atypical lymphocytes.

PB1987

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 HSCT.

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 HSC 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 MASSTotal mass score of 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 MASSTotal 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.

PB1988

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/109 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 Ampicillin-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, ceftazidime and ciprofloxacin and higher sensitivity was recorded in gentamicin and meropenem, Table 1 summarises the effectiveness of antibiotics used.

Summary/Conclusions: This study highlights 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.
Table 1. The sensitivity of antibiotic regimens used.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td></td>
<td>Neutropenia patients</td>
</tr>
<tr>
<td>Fluconazole + linezolid + gentamycin</td>
<td>99%</td>
</tr>
<tr>
<td>Micafungin + linezolid</td>
<td>99%</td>
</tr>
<tr>
<td>Liposomal amphotericin B</td>
<td>99%</td>
</tr>
<tr>
<td>Caspofungin + gentamycin</td>
<td>99%</td>
</tr>
</tbody>
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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 hypomelitamic agents to treat acute leukemia 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 Lymphoid AL and 94 were Myeloid AL. 41 patients were not eligible for intensive chemotherapy (for age and comorbidities) and were treated with hypomelitamic 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.Haemophilus 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 hypomelitamic 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.

Figure 1. Breakdown of mean 12 month resource utilisation costs for cohorts 1 and 2.
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.) adverse events. Renal toxicity of deferasirox is known to be reversibile; however, in patients with pre-existing compromise and those who concurrently take nephrotoxic drugs, treatment may be difficult to carry on.

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) and/or symptoms of IDA (anemia and/or symptoms of IDA) at health screening visit.

Results: Our cohort consisted of 58 patients, mainly affected by MDS (45 pts); other diagnosis were myelofibrosis (6 pts), NHL (2) and multifactorial anemia, not related to blood cancer (7). Only 38 out of 58 potentially eligible patients were assigned to iron chelation (see the Figure 1). The leading cause of ineligibility in our cohort was renal failure, while we had less difficulties in managing G.I. adverse events. Renal toxicity of deferasirox is known to be reversible; however, in patients with pre-existing compromise and those who concurrently take nephrotoxic drugs, treatment may be difficult to carry on.

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 centre actually receive and tolerate deferasirox treatment, among those potentially eligible patients.

Figure 1.

Summary/Conclusions: Our data are in line with literature. However, there is still room for improvement, especially in the category of non-MDS patients, who are often under-treated. Furthermore, the introduction of a new formulation of deferasirox, which is forthcoming, may hopefully reduce G.I. toxicity and improve tolerance and patients adherence to therapy.

PB1902
NONINVASIVE TRANSCUTANEOUS SPOT-CHECKING OF TOTAL HEMOGLOBIN FOR THE SCREENING OF ANEMIA IN CAMBODIAN CHILDREN FROM REMOTE RURAL AREAS
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Background: Previous studies have reported a high prevalence of anemia among school-aged children from Cambodia, ranging from 21 to 64%. Although iron deficiency accounts for the majority of cases, additional nutritional and non-nutritional etiologies have been identified. Children living in rural or remote areas, with limited access to health facilities, are at high-risk of developing anemia, and therefore, painless, fast, and reduced cost screening tests are needed.

Aims: The aim of our study is to evaluate the role of a portable device for transcutaneous noninvasive 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 ±0.39 g/dl (range 9-16 g/dl). Overall, the prevalence of anemia in the entire population was 34.5%. Anemia was present in 53/1 (16.1%) of the children within group 1, 97/189 (51.3%) 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: Taken together, 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.
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 cell 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 2016, all the consecutive samples (pediatric and adult) with anemia (as defined by WHO) which had sedimantation 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 hbf adjusted by age and sex, as defined by WHO. Ferritin 20-120µg/L and SR<20mm/h. ZPP measurement was performed by hematoclinometry (AVIV, 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 considered a p-value considerably significative at 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 in F [1.1-78], 28y in males (M) [1-78]; mean Hb was 10.6g/dL [SD 1.4]; mean ferritin was 9.3 ug/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 47y in F [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 21]. GC: 69.4% F; mean age 44.8y in F [1-79], and 37y in M [2-65 years]; mean Hb 13.8 g/dL [SD 0.9]; mean ferritin 71.9ug/L [SD 49.9] and ZPP 178.6 µmol [SD 26.8]; mean SR 14mm/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 (W=0.933) and 68% sensitivity and 70% of specificity to identify AID for ZPP ≥140 µmol (W=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 our 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 ZPP 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 underdeveloped countries.

PB1905

HYPERFERRITINEMIA AND SERUM INFLAMMATORY CYTOKINES IN ADULTS WITH NEWLY DIAGNOSED HEMOPHAGOCYTIC 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 lymphocyte 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 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 manifestation of an unknown malignancy, during progressive disease, or at malignancy relapse. The remaining 24% (17 patients) were also analyzed for cytokine storm. Serum ferritin concentration (ref.: 30–350 µg/L) at the time of HM-HLH diagnosis was elevated in all but one patient (70/71, 98%). Mean ferritinemia was 37,381±84,440 µg/L, median value 14,727 µg/L, and ferritinemia range 96–465,291 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 (79%) 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 whom 91% (63/69) had values ≥2400 U/mL. Moreover, in 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 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 83). 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.

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...
blood films are iron deficient pictures with the characteristic finding of reduced Hemoglobin (Hb), MCV and MCH. Above certain thresholds, the blood film adds little or no value to the CBC in these patients, apart from correlating with the iron studies results or suggesting iron studies when unavailable. One initiative used to manage the workload was based on this logic and aimed to reduce the blood film review rate using IT3000 technology (Roche).

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 ASYMETRIC DIMETIL ARGININE IN CHILDREN WITH IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA
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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 dimethil 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 anaemia group (p<0.05). There were no significant differences in ADMA level between groups. Any significant variety in CD105 EMP levels in the iron deficiency without anemia group was lower than the iron deficiency anaemia and control group; these levels in iron deficiency anaemia 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.

Results: We found a significant relationship between platelet count and serum 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 is a well recognized entity of the AOC, whereas reactive thrombocytosis in iron deficiency anaemia is less recognized. 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 underlying mechanism involving the tight regulation between iron metabolism and megakaryopoiesis in anemic patients.

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: This 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).
Myelodysplastic syndromes - Biology

PB1910

ROLE OF PRO-PHAGOCYTIC CALRECTICULIN 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 calrecticulin (CALR) and anti-phagocytic CD47, as they act in response to one another, reflecting a possible apoptosis vs survival mechanism in response to chemotherapies.

Aims: The aim of our study is to assess the changes in CALR and CD47 levels during treatment of MDS and MPN with azacitidine (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-dragging 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 cell model (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 9 fold increase in CALR and CD47 expression was seen, 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 lines 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/MPN models showed excessive rise in CD47 expression and low expres- sion 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 MYELODYSPLASTIC SYNDROME SUSCEPTIBILITY AND PROGNOSIS

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Background: Heterozygous deletion of RPS14 occurs in isolated interstitial deletion of chromosome 5q in patients with myelodysplastic syndrome (MDS). 5q- MDS has been linked to impaired erythropoiesis and it is characterized by 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- MDS patients. Recently, the pivotal role of RPS14 in human erythropoiesis during 5q- MDS pathology has been demonstrated. RPS14 haploinsufficiency promotes 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 poten- tially beneficed by lenalidomide therapy. In this work, we explore the origin of the altered 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 expres- sion 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 sup- pressor 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 beneficed 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 dif- ferences 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 profiles were 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 beneficed by lenalidomide therapy. Nevertheless, we did not observe a signif- icant increase in SPARC expression in RPS14 low expressing patients. Treatment levels of 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. Then, the origin of the reduced RPS14 expression was analysed using Ion Proton sequencing.

Summary/Conclusions: Although the important role of RPS14 in MDS pathol-
ogy has been recently demonstrated, the origin of RPS14 downregulation in about 50% of non-5q Patients remains unknown. Our results suggest that the origin of RPS14 decreased expression is not related to 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 and isolated del(5q). 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 had MDS-EB, 35 patients had MDS-MDL, 26 patients had MDS-SLD, 26 patients had MDS-SLD- RS, 12 patients had MDS with isolated del(5q), and 8 had complex karyotypes. Among the latter, 7 were associated with MDS-EB.

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 control. The median level of hemoglobin was 9.92±1.96 g/dl (p<0.0001), the median MCV (99.2±10.56 fl; p<0.0001), reticulocyte counts 44.3±10.71% (range 8-165.9; p=0.0001), immature reticulocytes fraction (IRF%) and the neutrophil median position on 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 had MDS-EB, 35 patients had MDS-MDL, 26 patients had MDS-SLD, 26 patients had MDS-SLD- RS, 12 patients had MDS-SLD without RS and 3 MDS with isolated del(5q). Sixty-two patients had a normal karyotype, 24 displayed abnormalities classically reported in MDS, and 8 had complex karyotypes. Among the latter, 7 were associated with MDS-EB.

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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 reseeded 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 ΔΔCt 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). Patients were stratified into risk groups according to IPSS and IPSS-R scores; survival probabilities were estimated using the Kaplan-Meier method.

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 80 g/L (29-150), more than 60% of patients had severe anaemia 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 by morphology FAB as RA (n=45), REAB (n=34), RARS (n=16), other (n=6). Classification by WHO 2008 included CRDU (n= 31 of which RA : 18, RT : 10, RN : 3), CRDM (n= 16), 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 anomaly (n=19), double anomaly (n=5) and complex (n=17). The main cytogenetic abnormalities seen were isolated 5q deletion (n=4), isolated 7q deletion (n=2), isolated 20q deletion (n=6), isolated trisomy 8 (n=2), 17p13 deletion (n=6), -Y (n=1), complex aberrations t≥3 (n=6), complex aberrations t≥5 (n=6), complex aberrations t≥7 (n=5), others (n=3). IPSS was assessed in 84 patients: 27% (low risk), 44% (intermediate 1), 24% (intermediate 2), 5% (high risk). IPSS-R was assessed in 84 patients; 18% very low risk, 30% low risk, 22.5% intermediate, 15.5% High risk, 14% very High risk). Leukemic transformation into AML occurred in 33% of patients in a median time of 12 months. According to IPSS, the median OS time survival is not reached for low risk group, 41 months (m) for Intermediate 1 risk, 11 m for Intermediate 2 risk, and 4 m for High risk. According to IPSS-R, the median OS time survival is not reached for Very low risk, 43 m for low risk, 24 m for Intermediate risk, 18 m for High risk and 4 m for Very high risk.

Summary/Conclusions: Our results are in agreement with those previously published regarding demographic features, distribution of recurring cytogenetic abnormalities and prediction of survival. Myelodysplasias are among the most difficult haematological 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 classify MDS is of an major importance. This is especially true in emerging countries where few therapeutic means are available, hence the need to predict the prognosis of these diseases in order to better target treatments. To the best of our knowledge, it is the first study conducted in our country.
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 years had >75% and 30%, respectively. The patients aged >65 received DNA hypomethylating agents (40%), anthracycline-cytarabine combinations (9%), low-dose cytarabine or hydroxyurea (17%), and supportive measures (34%). 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 allogenic transplantation, but 44% died over the induction chemotherapy in the group of anthracycline-araC combinations, 3.81 months in chemotherapy plus allogenic transplantation. The OS in patients aged >65 was 20.2 months in chemotherapy plus supportive measures group (Figure 1).

Figure 1.

Summary/Conclusions: The AML with MRC patients is a group with difficult treatment decisions and poor prognosis, in whom only the chemotherapy plus allogenic 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-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-T, and CMML), excluding 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.

Patients aged 1,200 and 1,800 mg were administered intravenously over 72 h, followed by 11-day monitoring in one 14-day cycle. 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 June 2012 and February 2015, 7 male and 2 female pts (median age: 70; range: 36-84) were enrolled, and 3 and 6 pts were eventu-

ally assigned to the 1,200 and 1,800 mg arms, respectively. According to the FAB classification, 6, 2, and 1 pts were categorized to RAEB, RAEB-T, and CMML, respectively. There were 1 pt each in Int-1-risk and high-risk groups, with 1 and 2 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 not in the 1,200 mg arm but in the 1,800 mg arm: 5 episodes of grade 3 non-hematologic toxicities in 2 pts. One pt developed grade 3/4 infections, grade 3/4 neutropenia, as well as 1 case of grade 3 hyponatremia. The other 3 episodes of hypochloremia, pustular rash, and hyponatraemia. Thus, 2 among 6 pts in the 1,800 mg arm developed DLT, which led us to conclude that 1,800 mg/day is the RD for Japanese pts. No deaths occurred during the study period. However, 5 pts died during follow-up, 4 of whom from primary disease progression. Furthermore, 1 pt died of grade 5 bacterial pneumonia that was rated to “Unrelated”. In the 1,200 mg arm, 2 cases each of grade 3 thrombocytopenia, grade 4 neutropenia, and grade 3/4 leucopenia, as well as 1 case of grade 3 lymphopenia developed. In the 1,800 mg arm, 3 cases of grade 3/4 leucopenia, 2 cases each of grade 4 neutropenia, grade 3/4 neutropenia, as well as 1 case each of grade 3 lymphopenia, increased C-reactive protein, erythrophagocytosis, and hypocholesterolemia developed. Three cases of SAEs, including grade 4 meningitis, grade 4 sepsis, and grade 3 catheter-related infection, developed in the 1,800 mg arm. Stable disease was obtained in 2 pts in the 1,800 mg arm. 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 6.74±2.39 μg/mL, respectively. The AUC 0-∞ values were 314.6±142.7 and 324.6±83.5 μg · hr/mL, respectively.

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 HAEMATOLOGICAL 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 long-term outcomes of higher-risk MDS patients and is now the reference frontline therapy of higher-risk MDS not eligible for allogeneic stem cell transplant. Anaemia is the most common symptom of MDS and most patients become transfusion-dependent with the risk of iron overload. Deferasirox is an orally available iron chelator administered once-daily in transfusion-dependent patients with various chronic anaemias. Its efficacy has been established in controlled clinical trials.

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/m2(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. A 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 2-4 year rate of prolonged complete response and 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 CYTOGENETIC ANALYSIS AT DIAGNOSIS PREDICT THIS RISK? 
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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 episodes and infections 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. Largely, studies have failed to predict this cohort of patients and little is known regarding identifiable risk factors.

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 228 of the above patients and univariate 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 autotransfusions] 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), C/D/Chido/Bga (1 case each). Collectively, alloantibodies against the Rh and Kell system were encountered in 69% of this cohort. 6 out of 8 patients with anti-C had also developed a second antibody. The most common antibody 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), C/D/Chido/Bga (1 case each). Collectively, alloantibodies against the Rh and Kell system were encountered in 69% of this cohort. 6 out of 8 patients with anti-C had also developed a second antibody. The most common antibody 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), C/D/Chido/Bga (1 case each). Collectively, alloantibodies against the Rh and Kell system were encountered in 69% of this cohort. 6 out of 8 patients with anti-C had also developed a second antibody. The most common antibody 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), C/D/Chido/Bga (1 case each).

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, cytogenetic, previous transfusions) showed a significant association with 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: AN INTERNATIONAL PERSPECTIVE EXPERIENCE
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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 reduced intensity 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 occurrence of alloageneic peripheral blood stem cell transplantation among the intensity conditioning patients (CR) 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 under went transplantation at the BMT unit at Nasser Institute during a period of five years. The presence of GVHD was evaluated by the RIC regimen used for high-risk population. The study took into account the risk factors of alloimmunization.

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 34.5±10.1 years. A total of 21 patients (41.2%) were low risk, 24 patients (46.1%) were intermediate group and 6 patients (11.8%) were high risk. The median time of follow-up was 3.5 years (range 12 months to 12 years). The rate of alloimmunization in our cohort of patients was 44%. The most common site reactions among the most commonly occurring non-serious adverse events were: skin, mucosal, and laboratory abnormalities.

Summary/Conclusions: The overall incidence of grade ≥2 skin reaction and any grade skin reaction in our cohort was 26.2% and 62.7% respectively. The rate of chronic skin reactions among the most commonly occurring non-serious adverse events were: skin, mucosal, and laboratory abnormalities.

PB1923
MANAGEMENT OF MYELODYSPLASTIC SYNDROMES WITH ERYTHROPOIESIS STIMULATING AGENTS IN REAL-LIFE EXPERIENCE: AN UPDATE FROM RECAMDS
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Background: Erythropoiesis stimulating agents (ESAs) are the frontline therapy for anemia in the earlier stage of the disease can delay the need for RBC transfusion, hypothesis by slowing the disease course. It's matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs.

Aims: Macroryctosis is one of the cytogenetic hallmarks of erythrophagia in MDS; an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non transfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian register, was performed.

Methods: 183 patients, treated with standard-dose ESAs, have been retrospectively analyzed (Table 1). Data analysis was performed, according to IWG 2006 criteria, at the baseline, after 3 and 6 months of continuous treatment, with a sub-analysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.3±1 g/dl, mean serum EPO concentration: 51 mIU/L, after a mean time of diagnosis from 6 months (t=1.188). The rate of ESAs response (EORR) was 83.6% (153/183), no difference among WHO and IPSS subgroups was found: 132/183 (72.1%) achieved response after 3 months of treatment, while other 21/183 (11.2%) after 6 months. 19 patients with stable disease (non-responders, according to IWG
PB1924

CHARACTERIZATION OF MYELODYSPLASTIC SYNDROMES WITH TRANSFORMATION TO ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Myelodysplastic syndromes are heterogeneous diseases with variable probability of developing a transformation to acute leukaemia. The vast majority of these cases present a transformation to acute myeloid leukaemia. We here describe a series of 4 cases of MDS/CMMML with evolution to acute lymphoblastic leukaemia. These events are very rare and are too date only published as single cases.

Aims: The aim of these study is to better define cases of MDS transforming to ALL.

Methods: We describe 4 cases of patients suffering from MDS who in the course of the disease presented with ALL. Three of these cases presented in 1 centre, 1 in the other. all cases were documented in a 17-year time span. We than performed a literature research including at the moment 37 cases of MDS transforming to ALL as described in case reports.

Results: Subtypes of MDS are varying from low risk MDS with deletion (5q) (del(5q)) to refractory anaemia with excess of blasts in transformation (RAEB-T), classified as AML in newer WHO classifications (2008 and 2016) and CMMML, classified as MDS/MPN nowadays. Even if MDS subgroups are manifold, cytophenic results are less so. Two of the 4 patients described demonstrated KMT2A rearrangements, 1 already at MDS presentation, the other at ALL presentation. One patient presented with del(5q). Of the 37 cases we identified in the literature, 7 presented with del(5q) and 2 showed with anomalies of the 11q23 locus.

Summary/Conclusions: These preliminary data can suggest that, in the majority of MDS patients responsive to ESAs, the increase of Hb concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAS last even when the expression of dysplasia progresses.

PB1925

IMMUNOSUPPRESSIVE THERAPY AS FIRST-LINE TREATMENT OF PATIENTS WITH PRIMARY MDS

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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 occurrence has been recognized in 2001. Some MDS subtypes which 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 MDS, revised 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 31.6, and RAEB in 15.8%. Hypoplastic form of MDS were diagnosed in 63.2% patients. The increased number of lymphocytes in the bone marrow of patients was 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 31.6% (variants RA and RCMD), complete remission in 36.8% (variants RA, RCMD and RAEB). There was no response to treatment in 21,1% of patients (variants RCMD and RAEB). Positve 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 biopsies (36,8%). Dependence of treatment efficiency and cytophenic 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 VD/VDR is BGLAP encoding for the non-collagenous protein osteocalcin (OCN) produced by osteoblasts and implicated in osteogenesis. Furthermore, it has been shown that OCN is expressed by activated hematopoietic stem cells in hematological malignancies.

Aims: We initiated an exploratory study, collecting patients' data on serum VD, and osteocalcin (OCN)-levels in 59 unselected patients with MDS, MDS/myeloproliferative neoplasm (MPN) and secondary acute myeloid leukaemia (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 chemiluminescence immunoassay. Intact OCN was assessed by ELISA in serum samples. Pooled data were used for the analysis. The results were compared between groups defined by severity of disease according to WHO classification and IPSS-R.

Summary/Conclusions: Median serum 25(OH)D levels were 18 ng/ml in “(very) low” (n=20), 23 ng/ml (RAEB-1/2, 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
Sixty-five children with JMML diagnosed between 2002 and 2016 strategies of JMML patients were collected retrospectively from pediatric hematology-oncology centers in Turkey were enrolled into the study. The median age at diagnosis was 17 months (range, 2-117 months). Splenomegaly was present in 92% of patients at the time of diagnosis. The median WBC, mono-
sis. Genetic and epigenetic abnormalities are at the core of myeloid neoplasias biological heterogeneity is reflected in the clinical course, ranging from an indolent disease to entities with high risk of progression to AML and dismal prognosis. The objetive of this study is to establish if there is an negative association between the percentage of precursor B cells (%PBC) at diagnosis in IPSS-R very low, low or intermediate MS. The objetive of this study is to establish if there is an negative association between the percentage of precursor B cells (%PBC) at diagnosis in IPSS-R very low, low or intermediate MS. The objetive of this study is to establish if there is an negative association between the percentage of precursor B cells (%PBC) at diagnosis in IPSS-R very low, low or intermediate MS. The objetive of this study is to establish if there is an negative association between the percentage of precursor B cells (%PBC) at diagnosis in IPSS-R very low, low or intermediate MS. 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patients. All patients required erythropoiesis stimulating agents and 9 patients received treatment with azacytidine (AZA) including all the int-1 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. Two patients with comparable risk and age, and who achieved similar responses did not show any relevant difference in survival. To this end, we conclude that the most frequently mutated genes were related to signal transduction pathways (n=11; JAK1, JAK2, NRAS, CBL, GATA2, SH2B3, SF3R). Patients with IPSS-R scores had significantly lower serum EPO levels (p<0.001; median 32.35 vs 42.70 U/L). Furthermore, patients with such mutations demonstrated a clear discrepancy in survival, with a median OS of 19 months for patients who progressed to low, intermediate and high risk. According to IPSS-R, the median OS time survival is not reached for low risk, 43 months for very low risk, 24 m for low risk, 18 m for intermediate risk, 11 m for very high risk and 4 m for very high risk.

Summary/Conclusions: Our results are in agreement with those previously published regarding demographic features, distribution of pre-existing 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 in the clinical practice is fundamental, in order to identify which patient is candidate for ICT: older age, comorbidities, poor tolerance and compliance.

PB1931
IS PRE-TRANSPLANT THERAPY A KEY FACTOR IN INFLUENCING POST TRANSPLANTATION RELAPSE INCIDENCE IN EXCESS BLAST MYELODYSPLASTIC SYNDROMES? A SINGLE CENTRE EXPERIENCE
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Background: The importance of pre-transplant disease burden in myelodysplastic syndromes (MDS) as a factor influencing post hematopoietic cell transplantation (HCT) outcome is an important argument of debate. It has been reported that relapse rate (RR) after transplant is reduced in patients entering HCT with a lower blast count. However, a recent meta-analysis of the IPSS and R-IPSS has reported a higher risk of relapse in patients with high IPSS-R scores, and the question remains open in the context of low and intermediate risk MDS.

Aims: Here we review our data to evaluate if the intensity of pre-transplant therapy may influence post transplant RR.

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 anthracbyline based, intensive chemotherapy (i.e.). 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/weekly for 7 days), as bridge to transplant.

Results: Among the 101 patients we performed 14 HCT (between June 2008 and September 2016) in patients with MDS in excess blasts. Median patient age was 63.5 years (range: 49-69), male/female 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 6 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 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.e. 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. relapsed, 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 MYELODYSPLASTIC 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.

Methods: Among these 101 pts, 58 were male with a sex ratio=1,35; range in age 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 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 cytogenotype into 2 categories: 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).

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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 DFO after a previous treatment with deferoxamine (DFO), while 9 pts (13%) received DFO and 3 pts (4.3%) received DFO after DFX due to contraindications to DFX or toxicity. Medium time from diagnosis to the start of ICT: 18 months. Median number of RBC transfusions before the start of ICT: 37.5. 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 ≥12 months, and 25 pts (36.2%) for a period ≥24 months. 27 pts (39.1%) 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 <1,000, and 48 pts (69.6%) a SF value <2,500. Adverse events possibly related to DFX were observed in 30 pts (43.5%): renal (increase of serum creatinine): 14 pts (20.3%) (grade ≥2: 2 pts; grade ≥3: 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, while 9 pts (13%) received DFO and 3 pts (4.3%) received DFO after DFX because of toxicity. Medium time from diagnosis to the start of ICT: 18 months. Median number of RBC transfusions before the start of ICT: 18 months. Median SF level pre-ICT: 1964 ng/ml; median SF after ICT: 675 ng/ml; median duration of ICT: 12 (range 1-230) months. 36 pts (52.2%) continued ICT for a period ≥12 months, and 25 pts (36.2%) for a period ≥24 months. 27 pts (39.1%) showed a drop of SF <500, 13 pts (18.8%) showed an increase of SF ≥500. 12 pts (17.4%) achieved a SF value <1,000, and 48 pts (69.6%) a SF value <2,500. Adverse events possibly related to DFX were observed in 30 pts (43.5%): renal (increase of serum creatinine): 14 pts (20.3%) (grade ≥2: 2 pts; grade ≥3: 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).

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 patients, removed from the clotting profile of the patient and the treatment regimen.
been implicated in putative downstream signaling of RAS, and may therefore provide therapeutic targets for the treatment 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 contribution to important signaling pathways which regulate MM cell survival and proliferation. Because oncogenic RAS is a potential activator of the Ral pathway via the RAF/MEK/ERK signaling axis and RalA, RalB is activated through non-kinase mechanisms, we investigated whether Ral pathway activation after Ral knockdown contributes to oncogenic RAS. Therefore, investigation of the functional network of Ral may be important to identify useful clinical targets. 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 and RalB was achieved through electroporation of MM cell lines and the effect on cell proliferation and apoptosis 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 isoforms 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 not impair MM cell survival in less than half of the cell lines. Western analysis revealed no alteration of classical RAF/MEK/ERK 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 WALDENSTRON MACROGLOBULINEMIA

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Background: Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytoid lymphoma with IgM monoclonal gammapathy. Most of WM harbor MYD88 L265P mutation. Therefore, investigation of the functional network of Ral may be important to identify useful clinical targets.

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 specific 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)] with WM, 10.8% (9/83) in patients with DLBCL, 9.5% (6/63) in patients with CLL, 0% (0/15) in patients with B-ALL, and 0% in 200 healthy persons. Among 31 WM patients, 10.9% (10/91) in patients with WM, 9.5% (5/63) in patients with CLL, 0% (0/15) in patients with 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 of the WM patients have CXCR4 mutation (10.8%), which may represent a rare mutation that occurs in WM patients. These findings were not formally reported. Since 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, anemia, hyperviscosity showed no significant difference among 3 groups. Group 1 showed adverse survival and 1 year survival rate of group 1 (66.7%) was lower than that of group 2 (90.5%, 94.7%), though it was not statistically significant (P=0.410). There were no death events in group 3 (MYD88L265P and CXCR4Mutations) patients 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 next generation sequencing can detect specific CXCR4 mutation in WM patients. Patients with MYD88WT and CXCR4WT showed higher IgM level and lower survival, suggesting an adverse prognostic implication. This is the first report on CXCR4 mutation in Korean WM patients.

PB1936

THE CLINICAL IMPACT OF CHROMOSOMAL TRANSLOCATION t(14;16)(q32;q23) IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Translocation t(14;16)(q32;q23) in plasma cells is considered as a strong negative prognostic factor in patients with multiple myeloma (MM). The oncogenic potential of this chromosomal aberration is based on the overexpression of the c-MAF protooncogene (located at 16q23) under strong enhancer of IgH gene (14q32). Although the IgH/MAF 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 IMWG risk classification system. The t(14;16) positive patients in our study are considered to have a high risk MM as having at least one of the following aberrations: deletion of 17p13 (TP53 gene), translocation t(14;16)(p13;q23) 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 survival (EFS) and overall survival (OS) in cohort of IgH/MAF positive MM patients in comparison with control group of 30 MM IgH/MAF 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 1q21 (RB1 gene/monosomy 13 (2), gain of 1q21/deletion of 1p33 (3) and deletion of TP53 gene (4). Cases with rearranged IgH gene were gradually examined for 3 specific translocations- 1) t(11;14)(q13;q32), 2) t(4;14)(p16;q32) and 3) t(14;16)(q32;q23). 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, p=0.285). Fourteen t(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 t(14;16) positive patients (83.3%), two or more additional high risk 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/MAF 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.

Supported by grants RVO-VFN64165, ProgresQ28 and GACR P302/12/G157.
PB1937
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 schedules, especially bortezomib or bortezomib-based treatment, is 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 nonneuronal 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 4 inducted in the VMP or VTD therapy. The control group consisted healthy age- and sex-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 significantly 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-κB during the treatment of MM, since reduction of NF-κB 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 GAMMAPATHIES
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Background: Monoclonal gammapathies (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 diagnosed by the clonal proliferation of plasma cells. MG is consistently preceded by a pre-neoplastic entity, called monoclonal gammapathy 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 monoclonal proteins and by the presence of so-called CRAB criteria and/or biomarkers of malignancy (as clonal BM plasma cells > 60%, 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: The study was designed to test the hypothesis that SNPs involved in NHEJ repair pathway (XRCC5, XRCC4) and in NF-κB pathway (NFKB2, and BIRC5) may have impact in MG susceptibility and prognosis.

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 MM 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 (CI95%) calculated by logistic regression analysis. We also investigated the association of these SNPs with overall survival through Kaplan Meier curves. All statistical analyses had a significance levels 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 70.11±10.25 years old. Among the individuals, 32% (20/63) were females and 68% (43/63) were males; the mean age was 69.90±10.06 years old. Most of 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 NFKB2 rs12769316 GA and GA genotypes (OR 0.346, 95%CI 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, 95%CI 5.06 199.70, p<0.01). BIRC5 GCC haplotype (rs4789551, rs9904341, and rs8073069) was found in one patient and absent in controls.

Summary/Conclusions: The present study suggests that NFKB2 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 77 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 cell lines. Knockdown 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 ENDONOUS RNA TO REGULATE PTEN 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 tumor suppressive 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.

haematologica | 2017; 102(s2) | 773
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 stage (II and III) and impaired renal function (eGFR) proteinuria. 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 subsequently inhibit MM cell proliferation and angiogenesis through sponging miR-181a in MM cells. Cytometric enrichment of PTEN by MF-CD45-TACs.

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 MYELOMA PATIENT WITH MICROWFLUIDIC 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-cell 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%±5.6% in the marrow. 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 diagnosis, the clinical outcomes of MM will be significantly improved.

PB1942

SERUM FREE LIGHT CHAIN RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: 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 gammopathy 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 Mprotein less than 30 g/L, bone marrow plasma cells 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 reference range of κ/ʎ 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 immunoelectrophoresis or immunofixation was done in 85 patients. Of these, The median serum M protein size at diagnosis was 12 g/L (1.7-28.5 g/L). IgG monoclonal - 68% (85 patients), 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 risk 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 protein (kappa or lambda). Our data support the hypothesis that an abnormal serum FLC ratio, non-immunoglobulin G (non-IgG) MGUS, and a high serum M protein level (>15 g/L) had a major risk of progression.

PB1943

INTENSITY OF EXPRESSION OF MULTIDRUG RESISTANCE GENES AFFECT ON THE OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA WERE TREATED WITH BORTEZOMIB AND ASSOCIATED WITH THE INITIAL MULTIDRUG RESISTANCE

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Background: Bortezomib is an important drug in multiple myeloma (MM) treatment, but the resistance to this treatment exist. Many conflicting data suggests that cellular overexpression of multidrug resistance (MDR) genes may reduce the effectiveness of bortezomib - containing treatment. The main indicator of the effectiveness of the treatment of MM is the overall survival of patients.

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. We studied the expression of the bone marrow cells (30 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 group of 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 electrophoretic bands) to 4 points (bright glow of the transcript).

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±0.2, with RR MM 1.9±0.3, p<0.05). The MDR 1 mRNA expression was 1.5±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 MRP 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 5 months and in those with low LRP expression was 8 months).

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 genetic abnormalities was performed by standard GTG-method and interphase FISH analyses with DNA probes: LSI 13(RB1), 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 -592 TT, IL-6 -174 GG and IL-6 n565 GG; gr. 1) as additional negative prognostic markers but IL-4 -33 CC and TGF-β1 c205 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).

<table>
<thead>
<tr>
<th>Genotypes with prognostic markers</th>
<th>Abnormal cytokine profiles</th>
<th>Normal cytokine profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α -889 TT</td>
<td>0.779</td>
<td>0.222</td>
</tr>
<tr>
<td>IL-1β -592 TT</td>
<td>0.317</td>
<td>0.999</td>
</tr>
<tr>
<td>IL-6 -174 GG</td>
<td>0.857</td>
<td>0.333</td>
</tr>
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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 cytokine profile (0.89) compared to MM patients with negative (0.22) or mixed (0.33) genotypes but normal cytokine 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 cytokine 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 -592 TT, IL-6 -174 GG and IL-6 n565 GG as markers associated with the presence of cytogenetic abnormalities in MM patient cells. However, IL-4 -33 CC and TGF-β1 c205 GG genotypes as additional positive 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. Some 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 the development of which play a role as environmental factors and genetic determinants. At present time, they are considered as real risk factors for the development of chronic lymphoproliferative disorders (CLD) and multiple myeloma (MM). It is noted that in the pathogenesis of hematological diseases 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 northeast 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%) six 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: IL-10 (rs1800871), IL-1β (rs886864), IL-4 (rs2243250), TNFα (rs1801274), 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-Dimensionality Reduction) [Liu X.Y. et al., 2007, http://www.healthsystem.virginia.edu/internet/addiction-genomics/Software/].

Results: In the analyzed group of patients with CLD and MM identified almost 78 753 combinations of multi-locus genotypes of the 13 immune response genes is 1 594 323 theoretically possible, indicating the non-random nature of the combination of allelic variants of analyzed genes. A statistically significant two-, three-, four-, five-, six-, seven- and eight-loci model of inter-gene interactions at the investigated hematological malignancies: - IL-4 (C-589T) and CD14 (C-159T) (χ²=8.39, p=0.0038); - IL-4 (C-589T) and CD14 (C-159T) and IL6 (C-174G) (χ²=12.14, p=0.0005); - IL-4 (C-589T) and IL1A (G-174A) and CD4 (C-282T) and IL2 (C-570T) (p=0.0001); - IL-4 (C-589T) and IL1A (G-174A) and IL10 (C-1081G) and CD14 (C-159T) and IL6 (C-174G) (χ²=16.88, p<0.0001); - IL-4 (C-589T) and IL1A (G-174A) and IL10 (C-819T) and TNF (C-308A) and CD14 (C-159T) and IL2 (T-330G) (χ²=16.98, p=0.0001); - IL-4 (C-589T) and IL1A (G-174A) and IL10 (C-819T) and TNF (C-308A) and CD14 (C-159T) and IL2 (T-330G) and IL1b (T-31C) (χ²=16.98, p<0.0001); - IL-4 (C-589T) and IL1A (G-174A) and IL10 (C-819T) and TNF (C-308A) and CD14 (C-159T) and IL2 (T-330G) and IL1b (T-31C) and TLR2 (A9753Gln) (χ²=16.98, p=0.0001).

Summary/Conclusions: The findings suggest an important role of immune response genes in the development of a number of chronic lymphoproliferative disorders and multiple myeloma, and can later be used as diagnostic and prognostic markers of different types of hematological malignancies. In addition, these polymorphisms may help to identify not only the genetic criteria for high and low risk of hematological malignancies studied, but also to determine their prognostic significance in the clinical course of these diseases.

PB1946
FEATURES OF STROMAL ELEMENTS IN HEMATOPOIETIC MARROW NICHE IN MULTIPLE MYELOMA
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Background: Structure of bone marrow stroma – mesenchymal stromal cells (MSC), endosteal stromal cells, and microvessels forming the hematopoietic niche and regulate the development of hematopoietic stem cells (HSC). Analysis of morphological changes of these elements of the hematopoietic niche is important to clarify the pathogenesis of multiple myeloma (MM). Aims: To investigate the morphological and functional characteristics of stromal elements of the hematopoietic niche in bone marrow of patients with MM, as well as the characteristics of culture of mesenchymal stromal cells (MSC) and hematopoietic stem cells (HSC).

Methods: 42 transplanted bone marrow from patients diagnosed with MM were used for the study. The age of the patients ranged from 53 to 72. The study applied histological, histochemical, immunohistochemical (IHC) and morphometric methods (VideoTest®). Also 20 patients from this group conducted cultural studies for the determination of colony-forming ability of HSC and morphofunctional status of MSC.

Results: Myeloma cellular composition of infiltrates were polymorphic. The surveyed patients were allocated to 3 types of infiltration: nodular, interstitial, diffuse. The histogenesis of infiltration was confirmed by IHC research with antibodies 79B, CD 138, CD 38.Regardless of the type of infiltration in all patients were revealed marked foci destructive changes of bone tissue. The density of microvessels N.Y. Semenova1,*, S. Bessmeltsev1, V. Rugal1

Summary/Conclusions: Analysis of parenchymal-stromal relationships in transplanted bone marrow of patients with MM evidence of their violation in the context of malignancy of lymphopoiesis, while cultural studies have shown a decrease of colony-forming ability of HSC and proliferative capacity of MSC. Results: Regardless of the prevalence of neoplastic lesions, and myeloma infiltration noted in vitro studies preliminary data on lack of differences in the phenotype of MSC bone marrow of patients with MM and phenotype from healthy individuals, but differences in the proliferative ability of the MSC of patients with MM.

PB1947
Abstract withdrawn.
Myeloma and other monoclonal gammopathies - 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.

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. Overall response to first line treatment was 70% for those with RI (CR 20%) and 68.8% in patients without RI (CR 15.4%). Treatment related adverse effects were higher in patients with RI (45% vs 28.2%), being polynuropathy the most common side effect. Patients with RI required more dose adjustments (40% vs 6.3%). Renal response: 50% reversed RI, 10% achieved renal PR and 40% renal CR, all before the 4th month from diagnosis; 77.8% started early treatment and 70% received bortezomib (bz). Patients whose RI did not reverse had had late initiation of treatment in 78% and 40% received bz. Six patients (30%) remained in chronic HD, all had late initiation of treatment. Two of the 6 patients who required HD at diagnosis obtained later independence; both received rz and one was consolidated with autologous stem cell transplantation. Impact of RI on OS and EM: median OS in patients with RI was not significatively different to that of MM without RI (35.3 vs 43.3 months, p=0.346). Patients without RI had higher OS compared to those who had reversible renal failure and those who never recovered (43.3 vs 12 months, respectively, p=0.031). OS was higher in patients with RI who received bz vs other therapeutic schemes (42.5 vs 25.8 months, p=0.137). With a mean follow-up of 26 months, mortality was 40% and 28.1% in patients with and without RI, respectively. EM were also higher in patients with RI at diagnosis (50% vs 22.5%). The main cause of death in both groups was infection.

Summary/Conclusions: RI was frequent in NDMM and was associated with advanced disease and higher tumor mass (>90% stage III Durie-Salmon and ISS3), revealing a late diagnosis. Prompt institution of treatment and use of bz relates to higher recovery of renal function with lower dialysis independence. Although treatment and dose adjustments were higher in patients with RI this was not associated with lower response to treatment. Reversal of renal failure associates with better OS, similar to those without RI at diagnosis. EM are more prevalent in patients with RI at diagnosis. Even when the number of patients is small, this real life data supports the need of planning local strategies that lead to early diagnosis and initiation of treatment, which are crucial to reduce morbidity and mortality associated to RI in NDMM.

PB1950

THE EXPRESSION OF THE TRYPSTATSE 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 supported 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 proinflammator 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 antiinflammatory drugs, corticosteroids or bisphosphonates. Serum 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 mean value of the three areas 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 myeloma tumor and progress 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.

Figure 1. Characteristics of patients and overall survival according to renal function.
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 (0.5–41.0) 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 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).

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PI (n=162)</th>
<th>IMID (n=74)</th>
<th>PI+IMID (n=51)</th>
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<tr>
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<tr>
<td>ISS stage</td>
<td>67 (42)</td>
<td>61 (83)</td>
<td>67 (100)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>35 (21)</td>
<td>36 (49)</td>
<td>45 (88)</td>
</tr>
</tbody>
</table>

The median duration of treatment (mDoT) was longer for patients on IMID (6.4 mo), but shorter for those on PI (4.2 mo) or PI+IMID (4.4 mo). In the PI cohort, carfilzomib had a shorter mDoT than bortezomib (3.5 vs 4.7 mo). Of 3220 total HCP visits, the most common type was clinic/physician office (2732, 85%), followed by hospitalization (210, 7%) and hospital outpatient (54, 5%). Mean per-1000 patients-per-month total visits were higher for PI+IMID (876) than for PI (750) and IMID (494). This remained true for clinic/physician office, hospital outpatient and home healthcare/other. Patients on PI had more visits for management of MM treatment-related events (16%) than those on PI+IMID (10%) or IMID (7%) (Table 1). Notably, among patients on PI, those on carfilzomib had high mean per-1000 patients-per-month total visits (827), with per-1000 patients-per-month emergency room visits (18) and hospitalizations (78) higher than any other treatment; 19% (175) of visits were made for management of treatment-related events.

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.

The median duration of treatment (mDoT) was longer for patients on IMID (6.4 mo), but shorter for those on PI (4.2 mo) or PI+IMID (4.4 mo). In the PI cohort, carfilzomib had a shorter mDoT than bortezomib (3.5 vs 4.7 mo). Of 3220 total HCP visits, the most common type was clinic/physician office (2732, 85%), followed by hospitalization (210, 7%) and hospital outpatient (54, 5%). Mean per-1000 patients-per-month total visits were higher for PI+IMID (876) than for PI (750) and IMID (494). This remained true for clinic/physician office, hospital outpatient and home healthcare/other. Patients on PI had more visits for management of MM treatment-related events (16%) than those on PI+IMID (10%) or IMID (7%) (Table 1). Notably, among patients on PI, those on carfilzomib had high mean per-1000 patients-per-month total visits (827), with per-1000 patients-per-month emergency room visits (18) and hospitalizations (78) higher than any other treatment; 19% (175) of visits were made for management of treatment-related events.

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-κ in patients with IgG-λ 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 immunoparesis (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
associated with other risk factors for progression. Severe suppression (>50% below 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 (28%) and it was significantly associated with both 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.

Figure 1.

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: Extramedullary disease is an uncommon manifestation in multiple myeloma (MM) and can either accompany newly diagnosed disease or develop with disease progression or relapse. Extramedullary 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 evaluated the impact of this disease features 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 progression of bone-related extramedullary-disease (bEMM) and its relationship with soft-tissue related EMM (sEMM) in MM patients in our institution.

Results: 42 bEMM and 42 sEMM patients treated at Our Department between 2007 and 2016 were included in this study. Of the first group 10 presented EMM at diagnosis and 32 at relapse as well as 7 and 35 respectively of the second series. 31 among eEMM were dead and 11 were alive, 20 of bEMM 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 and/or surgical biopsy was diagnostic in 82% of the patients. The treatment was heterogeneous and all patients had received either thalidomide or bortezomib in the first-line of therapy. We showed that sEMM cohort has a significantly poorer survival compared to bEMM patients (median OS from diagnosis of EMM of 13 versus 58 months, P<0.001). Finally, lung, liver (parenchyma-EM) and brain involvement was significantly associated with shorter OS when compared to skin and lymph node masses respectively median OS of 12 and 10 months versus 18 and 15 months P<0.001. Conversely among bEMM group there wasn’t a significant advantage of outcome regarding the different bones involved. Kaplan-Meier estimates were used for survival analysis. The differences between survival-survival 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-fracture. In our population we have a case of breast-plasmacytoma diagnosed accidentally after reconstructive breast-surgery, where Polymerase Chain Reaction of immunoglobulin gene-rearrangement in the breast tissue excised confirmed reconstructive breast-surgery, where Polymerase Chain Reaction of immunoglobulin gene-rearrangement in the breast tissue excised confirmed

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 newly diagnosed multiple myeloma (MM) patients. Patients may be stratified 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 significantly 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 regi- mens were administered to 12% and 28% of patients, respectively, whereas 60% of the patients received bortezomib (54%) or carfilzomib-based (6%) regi- mens as induction therapy. Thirty-eight percent of the study population under- went 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 the 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 (87% vs 60%) and 5yr-OS (95% vs 77%) was among sEMM patients who showed a better outcome 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 38 patients with score 0-1, i.e. patients achieving CR and receiving maintenance therapy (score 0) or achieving CR but not receiving maintenance (score 1); high-risk (HR) group, including 70 patients with score 2-3, i.e. not achieving CR, who underwent maintenance therapy (score 2) or not achieving CR and not receiving maintenance (score 3). Dynamic 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.

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 International Myeloma 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 (WB-MRI). The introduction of these biomarkers has been shown 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 (p<0.001 vs. both (ii)) & (iii) the added value of dynamic contrast-enhanced WB-MRI (DCE-MRI) 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 & WB-MRI (T1- (+/-)T2 & T2-weighted sequences, diffusion-weighted sequences & additional DCE-MRI 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 analyses were 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 aspirate & 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<0.001). 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 26.6% of cases. WB-MRI-positive was seen in 9 patients (36%). Progression was seen in 7/9 patients (78%) where only 1/16 WB-MRI-negative patients (6.3%) developed MM (p<0.001). Median TTP was 19.9 months versus not reached (p=0.011). No significant difference was seen between both groups (p=0.453). DCE-MRI was positive in 14 patients (56%) thus identifying 5 additional WB-MRI-negative patients with measurable bone marrow involvement. No significant difference concerning progression risk was however seen between WB-MRI-negative patients being DCE-MRI-positive (5/19, 26.3%) or -negative (14/19, 73.7%) (p=0.317). Median follow-up was 40 months (range 10-120 months). One patient developed 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 insufficiency was seen.

**Summary/Conclusions:** Our data shows that WB-MRI positivity was the most frequent biomarker in a routine clinical setting. WB-MRI positivity, according to IMWG-criteria, clearly identifies patients with an increased risk of progression as was already shown previously. Although increasing the sensitivity of WB-MRI, addition of DCE-MRI-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 expert 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 associated in general population, actors suggested VTE risk population, also to confirm the IMWG criteria in MM patients, 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 hazards analysis.

**Results:** The median age at diagnosis was 69 years (range 24-90 years), and 47% were males. The results concerning treatment are extracted from 374 patients who were symptomatic. In general, 85% of patients received IMiDs-based regimen. Among those patients, 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 (54%), followed by low doses of aspirin (13%) and anti-vitamin K (VKA) (6%). Median follow-up of IMiDs-based regimens was (range, 1-293) and VTE occurred in 11% of patients, with a median time from diagnosis of 10 months. IMiDs based-regimen demonstrated to be a risk factor associated with a higher risk for VTE: BMI ≥30 kg/m2, prior Stroke or TIA, prior malignant 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.
LONG TERM SURVIVAL OF IGM MULTIPLE MYELOMA AND WALDENSTRÖM’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 monoclonal gammopathy. IgM MM is a rare and poorly characterized disease.

Aims: The present 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 seven 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 Western Blot analysis. 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 of undetermined 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 in 3 (20%) patients 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 2 (13%) WM patients. Increased concentrations of involved monoclonal FLC and abnormal FLC κ/λ ratio in serum (by Freelite) in 75% of IgM MM patients. It was shown that IgM clonality in IgM MM and WM patients can be determined by using immunoglobulin heavy chain /light chain (HLC) immunoaassays- Hevylite. Immunofixation and HLC ratios were concordant in all assessed IgM MM and WM patients. In IgM MM patients 13 of 15 patients (87%) had decreased concentration of uninvolved polyclonal FLC and abnormal FLC κ/λ ratio in serum using HLC test - has prognostic significance. The evaluation of IgM HLC in 13 patients with IgM MM at diagnosis revealed a decreased concentration of uninvolved IgM (HLC IgM <0.33 g/l, HLC IgM <0.20 g/l) in 5 patients and normal values in 8 patients. Median overall survival in patients with a decreased uninvolved polyclonal IgM was 15 months and in patients with normal polyclonal IgM 55 months (p<0.01).

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 20% above 15 years. Suppression of uninvolved polyclonal IgM (detectable by using HLC test) at the time of IgM myeloma diagnosis is unfavorable prognostic factor.

MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW PLASMA CELL DISORDERS PANEL

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Background: The BD OneFlow solution for plasma cell disorders incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consor- tium standardized reagent system. The BD OneFlow solution enables reproducible identification and differentiation of distinct cell populations by combining stan- dardized assays, setup reagents, and protocols. The plasma cell disorders (PCD) panel is composed of the BD OneFlow PCST (Plasma Cell Screening Tube) and BD OneFlow PCD. BD OneFlow PCST helps differentiate normal plasma cell populations from those requiring follow-up. The BD OneFlow PCD classification tube helps differentiate abnormal from normal plasma cell popu- lations. The BD OneFlow PCD tube, when run in parallel with BD OneFlow POST tube, characterizes the abnormal plasma cell population for identification of plasma cell disorders.

Aims: The objective of this study was to compare the accuracy between the BD OneFlow PCD system and the EF liquid comparator system.

Methods: De-identified remnant human bone marrow specimens (n=48) were collected at two study sites and tested in an unblinded manner within 26 hours of draw. Specimens were simultaneously stained with BD OneFlow PCD and BD OneFlow PCD and the EF liquid comparator system. 26 patients were analyzed with analysis were performed on a BD FACSCanto II instrument using standardized acquisition and analysis templates in BD FACSDiva software. For qualitative endpoints, overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For accuracy quantitative endpoints (% positive plasma cell population), the slope, intercept, and 95% confidence limits of the slope from Deming regression were calculated for the BD OneFlow vs EF methods.

Results: The BD OneFlow PCD system is in 100% agreement (26 of 26) with the EF system in classifying patients as having normal plasma cell populations. The BD OneFlow PCD system is in 100% agreement (22 of 22) with the EF system in identifying patients with a plasma cell disorder. Furthermore, the BD OneFlow PCD system correctly identified 100% of patients who had a plasma cell dis- order based on clinical results.

Summary/Conclusions: The multisite evaluation between the BD OneFlow PCD and BD OneFlow POST and PCD tubes and the EF liquid reagent system was fully concordant in identifying patients with abnormal plasma cell populations. Additionally, all subjects identified as having plasma cell disorder based on clinical results were identified as having plasma cell disorder by the BD OneFlow PCD system. The BD OneFlow PCD panel is a fully standardized and validated assay system for aiding in the diagnosis of plasma cell disorders from bone marrow specimens.

PRACTICE GAPS AND BARRIERS TO OPTIMAL MANAGEMENT OF MULTIPL MULTIPLE MYELOMA PATIENTS: RESULTS FROM A MIXED-METHODS STUDY IN 8 EUROPEAN COUNTRIES

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1Department of Diagnostics for Hematology, 2Department of Transfusion Medicine in Warsaw as well as 15 WM patients investigated and treated at the same period of time at our hospital.

Background: Previous studies have identified gaps and barriers in Multiple Myeloma (MM) patient care, especially in relation to treatment decision making. However, further research exploring the gaps in practice, and barriers to optimal management of MM patients from the healthcare providers’ perspectives, would provide real-life recent evidence of

PB1959

PB1960
These new agents, with their own specific safety and side effect profiles, are likely to add to the challenges already experienced by health care providers in their management of patients with MM.

PB1961
THE EXPRESSION OF APRIL BY MULTIPLE MYELOMA CELLS AND THEIR ROLE IN THE EVOLUTION OF MULTIPLE MYELOMA
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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 pathologic monoclonal immunoglobulins. Inducible 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 for activation of myeloma cells is NF-κB. APRIL can directly activate the NF-κB and has been found by studies that are the most important factors for the survival of healthy and myeloma cells.

Aims: Aim of this study was the study of APRIL expression in myeloma cells in the bone marrow of patients with MM and their possible association with cell proliferation and survival.

Methods: We studied 42 newly diagnosed patients with MM, 19 women and 23 men, aged 64,1±10.4 years. According to the ISS stage, 14 were stage I, 11 stage II and 17 stage III. Regarding the type of paraprotein that had been found, 23 patients had IgG, 14 IgA and 5 IgM. Serum samples with 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. 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 cytoplasm 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 evasced as the percentage of neoplastic plasma cells and according to the intensity of staining in four-grade scale 0: negative, + weak, ++ moderate and +++ 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. BM infiltration Il=0.03% Ki-67%=0.01, IL-6<0.001, IL-6, p<0.001). Eventually APRIL correlated significantly with all measured parameters e.g. BM infiltration r=0.386, p<0.01, with Ki-67 r=0.390 p<0.01, 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.

PB1962
DEVELOPMENT OF SECOND PRIMARY MALIGNANCY AFTER TREATMENT WITH LENALIDOMIDE: A SINGLE CENTRE EXPERIENCE
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Background: Lenalidomide is a well-established and effective treatment for haematological malignancies particularly multiple myeloma (MM), but also lymphoma and myelodysplastic syndromes. It can be used as both a single agent and in combination with dexamethasone or other chemotherapy agents. A previous study in myeloma patients demonstrated an increased incidence of second primary malignancy (SPM) in patients treated with lenalidomide and dexamethasone (1.7%) compared to those treated with dexamethasone and placebo (2%-3%) [1 - 3].

Aims: We reviewed all patients treated with Lenalidomide in a single centre from January 2008 to May 2016 to establish the real-world of SPM.

Methods: A database of patients (n=137) treated with lenalidomide in the specified timeframe was created from pharmacy records. A search of the hospital’s patient management system was performed to identify: (1) type and date of primary haematological diagnosis, and (2) type and date of second malignancy based on pathology. An analysis of the data was performed to establish: (1) incidence of SPM, (2) latency between primary haematological malignancy and SPM, (3) latency between starting lenalidomide and SPM, (4) types and subtypes of SPM.

Results: The majority of patients were treated for Multiple Myeloma (67%). Other primary haematological malignancies included myelodysplastic syndrome (MDS), Non-Hodgkin lymphoma (NHL), and Idiopathic myelofibrosis (IMF). The incidence of SPM post-treatment with lenalidomide was lower (1.7%), compared to 2.7% in the general population [1 - 3].

Summary/Conclusions: Studies show the estimated incidence of SPM in MM to lie between 2%-10% over a 25 year period [5]. This study demonstrates a higher incidence (12%), however it includes patients treated for other primary haematological malignancies. This data demonstrates a similar incidence of SPM to previous studies (8%) post-treatment with lenalidomide [1 - 3]. Haematological malignancy was the commonest SPM however this differs from other studies in that it showed a higher incidence of solid tumors (40%) as compared to hematological malignancy [4].

PB1963
SOLITARY PLASMACYTOMA. A SINGLE-CENTRE RETROSPECTIVE STUDY
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Background: Solitary plasmacytoma (SP) is a rare neoplastic mass of monoclonal plasma cells that can either be localized in bone (solitary plasmacytoma of bone, SPB), or soft tissue (extramedullary plasmacytoma, EMP), without evidence of multiple myeloma (MM). The median age at diagnosis is 65 years. Some patients present a monoclonal band (MB) at diagnosis and a proportion progresses to MM. The low incidence of this entity has prevented reaching definite conclusions with regards to prognostic factors and treatment.

Aims: In this study we retrospectively analysed the clinical presentation, treatment and outcome of all patients with SP treated in our centre in order to establish relevant prognostic clinical features and management options.

Methods: Between 1985 and 2016, 27 patients with SP (20 SPB, 7 EMP) were treated in Ramon y Cajal Hospital (Madrid), with a median follow up of 8 years. The time to relapse, progression to MM or death was measured in months. The progression free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method. The progression of SBP was evaluated using the Log Rank test. Student’s t-test was used to compare the average age at diagnosis. To determine the association between the presence of MB or the subtype of SP, and progression to MM, we used Fisher’s exact test. All statistical analysis was performed with the software SPSS 24.

Results: The median age at diagnosis was 56 years (range 18 - 81). 51 (17%) for SPB, and 72 (±5) for EMP (p=0.05), with a male:female ratio of 2.4:1. The most frequent location was the axial skeleton (80%) for SPB, and the airway in the case of EMP (57%). In most cases, the initial symptom of SPB had been pain.
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 48, 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 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 Gammapathies (RMG). Our RSA uses patient and disease characteristics at diagnosis and at initiation of second-line treatment (2L) and provides similar outcomes independent of risk group. Use of our RSA at 2L would support physician decision making to improve patient specific care.

**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 MULTIPLE MYELOMA PATIENTS WITH RISS 2 DISEASE IS ASSOCIATED WITH WORSE PROGNOSIS AND ABOLISHED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION**

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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, present 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 higher levels of bone marrow infiltration, renal impairment, elevated β2-microglobulin and cytogenetic...
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 aHCT.

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. All samples were routinely tested for the presence of recurrent chromosomal aberrations, i.e. del 1p, amp q, del 6q, amp 11q, del 13, del 17, 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 creatinine, LDH or risk status. 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, aH SCT, 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 aH SCT and age. In patients not undergoing aH SCT 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 aH SCT. As previously reported aH SCT 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 be possible these patients should undergo aH SCT.

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: A nationwide, multi-institutional survey was performed in 2015 and 2016 to analyse routine practice for myeloma patients outside clinical trials in Germany.

Aims: We aimed to investigate implementation of autologous stem cell transplantation (ASCT) into treatment of patients with newly diagnosed or relapsed multiple myeloma (MM) in Germany.

Methods: The analysis is based on a database built from university hospitals (UH), community hospitals (CH), office-based hematologists (OBH). Anonymized data were collected online based on retrospective chart review. The survey was sent as an online survey via email, in return and by online personal checks. We investigated which institutions initiated treatment in patients with ASCT, which were the characteristics for patients not-considered eligible for transplantation, how stem cell mobilization was performed, how many patients dropped out before planned transplantation and what were the frequencies of tandem ASCT and ASCT for relapsed disease.

Results: Data from 515 patients from 51 centres were available for the first half of 2015 and from 867 patients from 52 centres for the first half of 2016. There were 40% (2015) and 32% (2016) pts considered as eligible for ASCT in 1st line. Although the proportion of patients older than 60 years was not significantly different between health care providers in 2015 and 2016 (2015: 47%UH, 60%CH, 49%OBP /2016: 54%UH, 56%CH, 47%OBP), patients were considered more often transplant-eligible in UH (2015: 49% / 2016: 53%) than in CH (2015: 29% / 2016: 21%) or OBH (2015: 45% / 2016: 26%). In first-line treatment, 52% of patients eligible for SCT received mobilization chemotherapy in addition to induction therapy. More than half of the eligible patients were treated with tandem ASCT in 1st line. In 2015, 8% of patients and 1% of patients in 2016 were considered eligible and were ultimately treated with ASCT for relapsed disease. The most frequent reason for transplant-eligible patients not receiving ASCT were withdrawal of patients consent (first-line: 18%, second-line: 39%).

Summary/Conclusions: With our current analysis of a nationwide survey performed with different health care providers in Germany we demonstrate that implementation of ASCT is strongly influenced by the institution initiating primary therapy. Age does not seem to impact usage of ASCT compared to concomitant disease or patients’ and doctors’ preferences. Patients receive two or three autologous transplants, enabling a possible tandem ASCT and ASCT for relapsed disease.

PB1967
MODIFIED HYPERCVAD VERSUS BORTEZOBIUM-HYPERCVAD IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is an incurable plasma cell malignancy, in which aggressive relapses 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-hyperCARD’ (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 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/exposed 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 del17p) were present in 8 and extramedullary disease in 13 patients overall. Randomized mod-CVAD 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 0.4mg continuous IV infusion every 24 hours on days 1-4 (mod-CVAD) OR bortezomib 1.3mg/m2 on days 1-4; 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 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.

Summary/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...
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 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 was calculated using the Kaplan-Meier method. Adverse events were recorded and evaluated using NCI-CTCAE v 4.0.

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 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 (≥PR) 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, 15.8-48.1 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 received 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 rate was 11.7% (14 patients). The overall incidence of grade 3-4 hematologic adverse events was 12.5% and thrombocytopenia was 9.2% in all grades. Peniculiasis (15.8%), fatigue (14.2%) and herpes infections (8.0%) 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

M. Moya-Arnao1,*, V. Cabanas-Perianes1, M.J. Moreno Belmonte1, M. Brequenghi1, M. Martinez Marin1, E. Ferrando Kaplan-Meier methods, 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: 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 1-4). 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 enoxaparine to bemiparin, maintaining same dose of lenalidomide. Neither treatment with esteroids or antihistaminic were administrated. Dystonias were reported in 23.1% of patients (grade 2), all of them disappeared after treatment with clonazepam without lenalidomide dose reduction.

Table 1.

PB1970

PROLONGED THROMBOPROPHYLAXIS IN PATIENTS TREATED WITH LENALIDOMIDE AND DEXAMETHASONE DOES NOT SEEM STRICTLY MANDATORY TO PREVENT LATE THROMBOVASCULAR 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 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: ≥PR 56%, 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 (43 pts) had 0-1 risk factor for VTE. Median duration of TP is 4.8 months (range

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.

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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 lenalidomide starting and VTE occurrence was of 12.2 months (range 1-88.2 months), with only one patient developing early VTE among our group. In detail we observed 10 deep vein thrombosis (83%), 1 pulmonary embolism (8.5%), 1 myocardial infarction (8.5%). Most of patients developing VTE had good disease control (ßerPR 83%, 10 pts). Concomitant adverse events (AE) was registred in 41.7% of pts (5/12). Most common concomitant AE were infections of respiratory tract (3 pts) and gastrointestinal AE (2 pts). The median number of risk factors for VTE in patients developing or not thrombosis was similar (2.5 vs 2.0, P=0.092).

Table 1. Baseline distribution of risk factors for thrombosis in the population on study.

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 antiplatelet 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, 67; 2-4, 58. We collected pretreatment parameter at diagnosis as follows: monoclonal protein type (IgG 60; IgA 32; IgD 1; BIP 30; non-secretory 2), light chain (kappa, 72; lambda, 52; unknown 1), hemoglobin level (mean 8.9 g/dL [range 5.8-15.2]), estimated glomerular filtration rate (eGFR) (mean 49.3 mL/min [range 3.6-114.2]), calcium level (mean 10.0 mg/dL [range 8.7-20.2]), albumin level (mean 3.4 g/dL [range 1.0-5.3]), beta-2-microglobulin (mean 5.1 mg/L [range 1.6-51.3]), involved/uninvolved serum free-light chain (FLC) ratio (mean 143.8 [1.83-21133]), cytogenetic abnormalities by using fluorescence in situ hybridization (FISH) (none, 53; t(4;14), 7; del(17p), 14; t(4;14) & del(17p), 5; t(4;14) & t(14;16) & del(17p), 1).

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, 62; 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 serum calcium level greater than 11 mg/dL (HR 3.036, 95%CI 1.412-6.529) (Figure 1C). Among 80 patients with FISH data, survival of those with t(4;14) or del(17p) or t(14;16) was not statistically different (P=0.394). 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.

Figure 1.

Summary/Conclusions: Renal dysfunction and hypercalcemia at diagnosis is predictive of poor OS for elderly NDMM patients in real world.

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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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 work up and starting the therapy (n=121). 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 3.9% of them from 2015. Median age of patients at presentation is 56 years old, 3.33% between 30-40 years old, 18.33% between 40-50 years old, 35% between 50-60 years old, 31.67% between 60-70 years old and 11.67% between 70-80 years old. Male to female ratio 1.75:1 (Table 1). According to ISS stage patients were categorised into4% stage I, 31% stage II, 47% stage III. Restaging using the R-ISS revealed10% stage I, 26% stage II, 56% stage III. Almost half of our patients are diagnosed in the third stage, and more patients were shifted from stage I or II were categorised in the third stage due to either high LDH level, high cytogenetic risk or

haematologica | 2017; 102(s2) | 785
even both. First line treatment 55% of the patients received Bortezomib based triplet therapy 22% received CTD (Cyclophosphamide, Thalidomide, Dexametha-
sethane), 7% RD (Lenalidomide, Dexamethasethane), 3% CyBORD (Cyclo-
phosphamide, Bortezomib, Dexamethasathane), 3% RV (Lenalidomide, Bortezomib),
2% Thal-Dex (Thalidomide, Dexamethasethane), 2% RT (local Radiotherapy), 2%
WatchfulWait, 1% MP (Melphalan, Prednisone) and 3% refused for treatment and
lost follow up.

Table 1.

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Summary/Conclusions: Applying the RISS system to myeloma patients is a
significant 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.

PB1974

EPIDEMIOLOGY OF MULTIPLE MYELOMA. THE GRANADA MYELOMA
REGISTRY

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Background: The Granada Myeloma Registry is the second largest single-
institution population-based registry (Ríos-Tamayo et al, 2015) of multiple
myeloma (MM) referenced to date. Here we update and point out the epidemi-
ological 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 diag-
nosed with MM at our institution have been registered, including clinical, bio-
logical and socio-demographic variables, as previously reported. A com-
prehensive approach to comorbidty was recorded as well as diagnostic and treat-
ment 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: 12-93). The race was Caucasian in 98.9%.
In relation to occupation, 18.4% were skilled or elementary agricultural
workers. Only 9% 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 10.8%, Non-secretory 3.7%. The median overall survival for the subgroup of
patients presenting with plasmacytoma or smoldering disease was 6 months.

PB1973

FEASIBILITY/PHASE II STUDY OF MYELOABLATIVE BEAM ALLOGENIC
TRANSPLANTATION FOLLOWED BY ORAL IZXAZOMIB 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 bortezomib 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 patients (age >18, bortezomib naïve) were treated with a two-
HCT, bortezomib and an immunomodulatory agent; one of the following:
high risk criteria: deletion (del)17p, t(4;14), t(14;16), t(14;20), amp1q gain or del1p,
del13q by conventional karyotyping, hypodiploidy, high-risk GEP, B2M >5.5mg
plasmablastic morphology (>2%). At study entry 2 subjects were BMI <18.5,
were obese at the moment of diagnosis. 8.2% had other previously known or
synchronous neoplasm. 150 patients (30.1%) had three or more comorbidities.
Overall survival (OS) was estimated in 606 cases: 151 of them (24.9%) received a first autologous transplant. Median OS for the whole
cohort was 4.1 and 22.4 months for patients younger than 65 years or 65 years
and older, respectively (p<0.001). For patients diagnosed in 2010 or
later, 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 neoplasm. Infection is the
leading cause of death. Information derived from population-based registries
may help to complement data from clinical trials.
re-escalated back onto dexamethasone and alkylator (IV/oral) based regimes of 2nd and 3rd line IMiD therapy that these salvage regimes are highly effective. Patients on lenalidomide monotherapy post triplet/duplet induction were often of patients were prescribed lenalidomide 25mg od. Despite this 17 patients (36%) The longest observed PFS in the local cohort was 53 months. The average number of age, 50% reached cycle 26 compared to the national average of 16%. Average malformities] were identified in 9/46 and 16/46 were high-risk based on biomarker High risk cytogenetics [17p-, t(4:16), t(4:20), hypodiploidy, chromosome 1 abnormal- IgA 11/46, light chain disease 4/46 and 3 with IgD and non-secretory myeloma. Initial myeloma diagnosis was 71 years, with median age at starting lenalidomide and prolonging PFS. All continued to maintain their response. The median progression of which 5 (83.3%) were refractory to a PI. 1 patient discontinued due to personal choice. The overall response rate (ORR) was 70.5% (95% CI 64.1-76.5), 1 patient experiencing grade 4 anaemia. This resulted in Ixa dose reductions in 4 (16.7%) patients. Ixa was stopped in 1 patient due to adverse events. Summary/Conclusions: This real world dataset highlights differences in patients treated in routine practice to trials. No patients were treated at first relapse due to funding restrictions, whereas most in the trial were. Patients had up to 5 prior lines, all had prior PI exposure and a higher proportion were PI refractory (33% vs 2%) which correlated with a worse outcome. Nevertheless the overall efficacy of our study (ORR 70.8%; median PFS 19.23 months) was comparable to the TOURMALINE-MM1 trial which had an ORR of 78.3% and median PFS of 20.6 months in the Ixa group.

PB1976
EFFICACY AND TOLERABILITY OF LENOLIDOMIDE 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 often added to previous regimens to improve responses. The purpose of this study was to document the efficacy and tolerability of these drugs in a real world cohort to provide data for future comparison. Methods: Medical records of chemotherapy cycles were retrieved from local pharmacy data and national averages from Celgene ePAS 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-, (14q, 16q), (14q, 20q), hypodiploidy, chromosome 1 abnormal- als] 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 duets, thalidomide combinations, bortezomib-based therapy and autograft. National average for the% of patients reaching cycle 26 was 16% com- pared to our cohort reaching 31%. This included 10 patients receiving Ixa and 2 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 (inflection, cytopenias, live and death, progressing disease, other). Cytophenias or infections were seen in 45% of local patients with 28% of patients having subsequent dose reductions. Based on performance status, renal function and prior drug tolerability, only 30% of patients were prescribed lenalidomide 25mg od. Despite this 17 patients (36%) achieved a prolonged PFS of >20 months and 13/46 (30%) a PFS of >30 months. The median time on treatment in the local cohort was 53.5 months. The average number of cycles in those who progressed to pomalidomide was 12 (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 duets or triplets).

Summary/Conclusions: We conclude from this real-world retrospective review of 2nd and 3rd line IMiD therapy that these salvage regimens are highly effective. Patients on lenalidomide monotherapy post triplet/duplet induction were often re-escalated back onto dexamethasone and alkylator (IV/oral) based regimens 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
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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. Studies have shown that in ASCT for multiple myeloma, conditioning regimens containing busulfan is highly effective as HDM. Aims: Evaluate the safety and efficacy of BUCY (busulfan and cyclophos- phamide) conditioning regimen for autologous hematopoietic stem cell trans- plantation (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 mel- phalan. Those were compared between the two groups including the complica- tions, 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 transplantation-related adverse events were similar in both groups but the incidence of pulmonary infection and 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%. Correspond- ingly, 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 ine- rior to HDM regimen. In conclusion, BUCY regimen may replace HDM regimen as a standard conditioning regimen for ASCT in multiple myeloma.

PB1978
MULTIPLE MYELOMA WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT, 12 CASES AND REVIEW OF THE LITERATURE
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Background: Central nervous system (CNS) propagation is a rare event in multiple myeloma (MM), but may become more prevalent as newer treatment options allow patients to have a prolonged life expectancy and with this come the selection of increasingly aggressive clones. Aims: We reviewed 12 MM cases with CNS involvement treated in two hospitals. Methods: Statistical analyses were performed using the SPSS (version 20.0) software package.

Results: Between 2008 and 2015 twelve MM patient developed CNS involve- ment which presented in all cases at relapse. The median age at diagnosis and at CNS presentation were 55.7 and 58.5 years. At first presentation nine had ISS 3, one ISS 2 and two ISS 1 stage disease, two patient presented orig- inally with grade three plasmacytoma cell leukaemia. FISH showed 1q amplification in 4, 13q deletion in 4, 14q deletion in 4, translocation (4;14) in 1, (11;14) together with 17p deletion in 1, hyper- diploidy in 1 and complex karyotype in 2 cases. In 2 cases we demonstrated the development of new karyotypic abnormalities (one 1q amplification, one 17p deletion) at CNS progression. The median number of treatment lines prior to CNS progression was 4 (range 3-6). CNS involvement seen in 11/12 cases and in all but one cases, two patients had lenalidomide. Six patients had ASCT before the CNS progression from which one had a second ASCT and one a reduced intensifier allogeneic transplantation. The median time from diagnosis to CNS
progression was 23.9 (3-65) months. Eight patients presented with cerebral nerve palsy, 2 with paraplegia, 1 with hypothyroidism 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, intrathecal 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 776 days), both responded in terms of CNS symptoms to imid-based combination therapy and one had cranio-caudal radiotherapy (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 diseases 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 managed care, 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 pathology 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 Dara 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; Pre and Post baseline samples help to identify the Darzalex Band in the serum; our lab use a Sebia capillary 2 analyser to separate the Dara band for accurate reporting. Bone Marrow Testing: Daratumumab affects Immunophenotyping by masking the CD38 epitope used to identify plasma cells by flow cytometry; special kits are available using a different CD38 epitope thus dealing with this issue. Infusion Related Reactions (IRRs) have been reported in over half of patients receiving Daratumumab: 96% of these were seen at the first dose. Typically involving the upper respiratory tract and include rhinitis, cough, wheeze, bronchospasm, laryngospasm and chest pain. More rarely they include rash, fever, and nausea. Reactions can be grade 1-4 so it’s important that the patient is closely monitored where there is quick access to specialist staff, resuscitation equipment and respiratory support in a high dependency setting. Staff training is important as patients need to be aware that they report all new symptoms so the infusion is interrupted immediately and the IRRS treated and re-started at a lower rate when the symptoms have resolved. Premedication is given one hour prior to infusion and patients with a history of COPD receive extra support. Patient characteristics. Total:15. (Table 1).

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 considered not to be associated. Recent 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 level 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 progres- sion free survival of HIV+MM patients on HAART patients are not superior to that of HIV-negative MM patients. The survival of the HIV+ MM patients were also 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 osasialgia 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 mkmol/l. HIV and hepatitis C (genotype 1a) screening test were positive, confirmed with Western blot analysis. The CD4 count was 290 cells, HIV viral load 13,000 copies/ml, hepatitis C viral load 14.2 mln copies. He was started on HAART, combined with chemotherapy 5 courses of CP+CVP+MP and 7 V-MP. In 2017 total serum protein– 97.3 g/l, M-protein 31.2 g/l, serum creatinine 63.0 mkmol/l. Now he is active without any bone pain receives Pegasis 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

Table 1

<table>
<thead>
<tr>
<th>Age (years) at Diagnosis</th>
<th>Gender</th>
<th>Number of prior treatments</th>
<th>Regimens</th>
<th>Disease Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median: 62.3, IQR 46-76</td>
<td>Male: 8</td>
<td>Single agent: 1</td>
<td>Dara with Lenalidomide: 4</td>
<td>Palmar: 1</td>
</tr>
<tr>
<td>Range: 37-84</td>
<td>Female: 7</td>
<td>Dara with Bortezomib: 1</td>
<td>1-2</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.
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>
<thead>
<tr>
<th>Characteristics of patients with HIV infection and MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>M-Gradient</td>
</tr>
<tr>
<td>G-CSF admission</td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>PR</td>
</tr>
<tr>
<td>NSD</td>
</tr>
<tr>
<td>CD138/cytKappa.</td>
</tr>
<tr>
<td>PR patients (n=31) correlated with the serum M-gradient concentration (rs=0,42; p=0,019) versus 1,3%</td>
</tr>
</tbody>
</table>

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 pleraxifor was required in 27.8% of patients on high dose cyclophosphamide, as compared to 13% of patients on the lower dose regime and none of patients mobilized with GCSF. Addition of pleraxifor was required in 27.8% of patients on high dose cyclophosphamide and 23 patients with low dose cyclophosphamide during a similar period.

Summary/Conclusions: All 3 strategies for stem cell mobilization have their disadvantages such as febrile neutropenia.

PB1983

AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY MULTIPLE MYELOMA PATIENTS


Background: Autologous stem cell transplantation (ASCT) is currently approved as a “gold standard” first line treatment for multiple myeloma (MM) patients (pts) under 65 year old but the procedure could also be considered feasible in fit elderly patients based on several retrospective studies. The aim of our study was to retrospectively evaluate the tolerability and the efficacy of high dose chemotherapy followed by ASCT in selected ≥65 year old MM population.

Methods: We retrospectively analyzed consecutive MM pts aged 65 or older who underwent upfront ASCT at our institution from January 2009 to November 2015. Inclusion criteria for induction therapy included treatment with proteasome inhibitors and/or immunomodulatory drugs (bortezomib and/or thalidomide based), followed by high-dose cyclophosphamide plus G-CSF and subsequently underwent peripheral blood stem cells (PBSC) collection.
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 dexamethasone, VD, 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). PBSCT 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 administrated 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. At 2 years, 14/28 pts achieved VGPR, 7/28 pts in PR, 10 pts were in stable disease and 1 pt in progressive disease. At 3 years, 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 GAMMOPATHIES 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) and that most of these patients have no evidence of either gammopathy or disease. MGUS is a premalignant disorder characterized by the presence of a monoclonal component in the serum or urine that does not meet the criteria for multiple myeloma (MM). MGUS is easily detected in laboratory tests and should be monitored because 1% of MGUS per year progress to Multiple Myeloma (MM). 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 or immunofixation, allowing them to support the diagnosis of MGUS that years before remained undiagnosed.

Aims: The aim of this study is to determine the incidence of MGUS, MM and its different types in the reference population of a tertiary hospital in southern Spain between 2003 and 2015.

Methods: In a retrospective study, we determined the total number of MG and its different types diagnosed in our hospital 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 480,851.

Results: Results in Figure 1.

Summary/Conclusions: The aging of population and the higher sensitivity of laboratory techniques for diagnosing MG is 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 ≥2x109 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 74x109/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%) were submitted, at least, to one autologous stem cell transplant (ASCT). 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 ≤100x109/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 cannot 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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1Hematologia, Hospital universitario de Guadalajara, Guadalajara, Spain

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-Dexa-cyclophosphamide, and Poma-Bortezomib-dexa, ORR 38.9, 64.7 and 85%; PFS 4.4, 9.5, 10.7 months respectively).

Aims: To evaluate the response for treatment 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 were administered. We have had poor response in many cells 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
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 2005 to 2015.

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 male/female patients was similar between the AMR and MRDR (m:f 56%:44% and 61%:39%, respectively). PRESENTATION: IgG myeloma was the most common sub-type of disease in both 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% vs 23%:20%) 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 2.34:2.28 vs 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 (m:f 81%:78% vs 81%:78%, on the AMR and MRDR, respectively).

Figure 1. First line therapy was predominantly bortezomib (Velcade - V) based on both registries (81% vs 85%). V/dexamethasone (D) was the most common on the MRDR (29%) followed by V/thalidomide/D (VTD) (25%) with V/lenalidomide/D (VLD) 13% on the MRDR. 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.
LB1990

EARLY MORTALITY (<6 M) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: COMPREHENSIVE INTERVENTION

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1Hematology, Complejo Asistencial Universitario de León, LEON, Spain

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 real-world elderly patients exceeding 20% (1). The main cause of death is infectious complications (avoid unnecessary hospitalization, antibiotical prophylaxis) and rapid access to optimal antiMM treatments.

Aims: To promote the ambulatory regimen 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 years and/or renal failure with Septrim®.

Methods: Patients with MM in the 3rd period (2012-2015) were compared with those in the 2nd period (2008-2011). The median age at dx was 74 years (39-100). The number of patients <6m was 77. 60 died before 6 months: 55 before 2013 (29 in the 1st period, 46%); 15 after 2013 (12 after the 2nd period, 83%) (P<0.001).

Results: 22 of 26 in the second). Severe pneumococcal infections were infrequent (11%)
In the 3rd period, mortality <6m was reduced by 77% (22% vs 5%) (p=0.001); There was only 1 severe infection (OS) in this period (CMV reactivation, probably Pneumocystis pneumonia and E. Coli bacteriemia and an intestinal necrosis after an atrial fibrillation embolism) Figure 1 (upper corner). Improvement in early mortality increases significantly overall survival: 32.5 monts vs not reached pre and post-2013 (p=0.0034).

Summary/Conclusions: Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potentially 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, antibiopathly) and rapid access to optimal antiMM treatments. These improvements of short-term

Figure 1. (large graphic; legends: red: pre2013; blue: post2013).
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) were significantly different between the two groups (PFS, median 12.3 vs. 17.8 months in VMP and MP/CP group, respectively, P=0.018; OS, median 24.9 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.

**Methods:** From patients who were HIV-positive and on HAART undergoing ASCT for treatment of MM between January 2000 and June 2016 were collected and analyzed. **Aims:** The purpose of this case-control study was to compare the efficacy of VMP and MP/CP regimens for patients with HIV/AIDS. It stands to reason that MM patients with HIV on HAART may benefit equally from aggressive combination treatment of chemotherapy and ASCT.

**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. **Methods:** A single center retrospective case-series study was performed. **Results:** The following Table 1 lists patient characteristics. All were male with average age 53.2 years. All were diagnosed with HIV prior to diagnosis of MM and were appropriately treated with HAART prior to ASCT. All patients had received standard chemotherapy and ASCT. The HAART regimen was continued during ASCT. Patients experienced usual ASCT toxicities including diarrhea, mucositis, and neutropenic fever. One patient developed sepsis and small bowel obstruction, which resolved with antibiotics and conservative management. All patients had normal neutrophil and platelet engraftment. Post ASCT responses were complete remission (2 patients), very partial remission (1), partial remission (1) and minimal response (1). All patients are currently alive without relapse or progression 1-4 years from ASCT and receiving post ASCT maintenance with lenalidomide.

**Table 1.**

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**PB1994**

**EFFECTICITY OF AUTOLOGOUS STEM CELL TRANSPLANTATION FOR THE TREATMENT OF MULTIPLE MYELOMA IN HIV-POSITIVE PATIENTS – A CASE-SERIES**

**Background:** While hematopoietic malignancies are found at increased rates in individuals with acquired immunodeficiency syndrome (AIDS), the experience of multiple myeloma (MM) and human immunodeficiency virus (HIV) is less common, leading to a paucity of expertise in the treatment of individuals with these co-morbid conditions. Prior to the advent of highly active retroviral therapy (HAART), autologous stem cell transplant (ASCT) was relatively contraindicated for MM patients with HIV due to issues associated with stem cell harvest and the risk of opportunistic infections. With the widespread use of HAART for control of HIV, high dose chemotherapy and ASCT is now the preferred treatment for relapsed lymphoma, the leading hematopoietic malignancy associated with HIV/AIDS. It stands to reason that MM patients with HIV on HAART may benefit equally from aggressive combination treatment of chemotherapy and ASCT.

**Aims:** This study seeks to evaluate the clinical course and treatment outcomes of patients with HIV and MM treated with high dose therapy and ASCT.

**Methods:** A single-center retrospective case-series study was performed. Data from patients who were HIV-positive and on HAART undergoing ASCT for treatment of MM between January 2000 and June 2016 were collected and analyzed. **Results:** The following Table 1 lists patient characteristics. All were male with average age 53.2 years. All were diagnosed with HIV prior to diagnosis of MM and were appropriately treated with HAART prior to ASCT. All patients had received standard chemotherapy and ASCT. The HAART regimen was continued during ASCT. Patients experienced usual ASCT toxicities including diarrhea, mucositis, and neutropenic fever. One patient developed sepsis and small bowel obstruction, which resolved with antibiotics and conservative management. All patients had normal neutrophil and platelet engraftment. Post ASCT responses were complete remission (2 patients), very partial remission (1), partial remission (1) and minimal response (1). All patients are currently alive without relapse or progression 1-4 years from ASCT and receiving post ASCT maintenance with lenalidomide.

**Table 1.**
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 mostly 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 400 MBq of FDG. Images were analyzed using an iterative thresholding algorithm that delineates a continuous region based on Hounsfield units from the CT data (OsiriX 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 SUV measure that considers all bone marrow involvement. Global SUV/mean 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 SUV/mean uptake decreased after initiation of treatment in 17 (73.9%) of the cases and increased in 6 (26.1%) of the cases. In the patients group with extramedullary disease. Global assessment rather than focal analysis of discrete lesions represents a robust and straightforward method of determining total disease activity that potentially will be of value in treatment evaluation, disease monitoring and prognostication in multiple myeloma.

PB1996

VALUE OF MYELOMA 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 therapy.

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 bortezomib based 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 (IRSS-R) and Durie Salmon staging were calculated. Patients with other malignant diseases as revealed by the observed statistical outcomes were overall survival (OS) defined as death from myeloma or any other cause and time to next treatment (TNT), defined as time from transplant to next new therapy or death of any cause.

Results: From January 2012 to December 2016 hundred and one patient with MM (49 male, 52 female), median age 55 (range 22-71), were transplanted. Bortezomib based induction therapy was used in 55 (54.5%) and VAD induction was used in 46 (45.5%) patients. Median OS of all treated patients was 73 months; median OS of VAD group was 73 months while in bortezomib group median OS was not reached, but this difference was not statistically significant (p=0.19). TNT was significantly longer in bortezomib group than in VAD one (27.8 vs 17.5 months respectively; p=0.02). Interestingly prognostic indices could not discriminate patient groups according to OS (p=0.1), but could discriminate them due to TNT (p=0.008), possibly due to cross-over to bortezomib treatment after treatment failure. TNT had a significant correlation with levels of LDH (p=0.04) and no significant correlation with number of plasma cells in bone marrow. OS was significantly longer in those with longer duration of time to next treatment (p=0.004). There was no difference in OS or TNT in patients treated with tandem transplant vs single transplant (p=0.68 and p=0.57 respectively), possibly due to heterogeneity of tandem group.

Summary/Conclusions: Evaluation of novel drug therapy seems to converge risk groups to lower ones, prognostic indices remain relevant. Due to heterogeneity of patients and myriad of known prognostic factors further studies are needed so they may be translated into risk adapted therapy approach.

PB1997

WHICH ORGAN SHOULD WE BIOPSY TO DIAGNOSE AL AMYLOIDOSIS?

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Background: Light chain (AL) amyloidosis is a deposition disease with can affect many organs and with a variable but usually bad, prognosis. Therapy requires a quick and correct diagnosis. Accurate identification of amyloid deposition and of the amyloid subtype in tissue biopsies is thus, mandatory. Random 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 nephritic 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-TTR 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.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Biopsies</th>
<th>Alamyloidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>59</td>
<td>25 (42.5%)</td>
</tr>
<tr>
<td>Intestine/Rectum/ Stomach</td>
<td>10</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Gut/Intestine</td>
<td>12</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>11</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Skin</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Tongue</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>3</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>(Salivary gland/ Muscles)</td>
<td>3</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>(Salivary gland/ Muscles)</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>(Salivary gland/ Muscles)</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Total of biopsies</td>
<td>152</td>
<td>95 (62.5%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Prognosis in AL amyloidosis is slowly improving with the use of new anti-myeloma drugs and may improve further with new 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 organs such as skin or rectum should be discouraged.
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 in the Cyclo group, (HR 0.99; CI 0.42 – 2.34; p=0.99). There was no significant difference in overall survival 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 to 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 (Ld) 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 AUC0-24 of Len was 2032.8ng•hr/ml (sensitivity 86.1%, specificity 87%) and non-hematologic AEs 3023.8ng•hr/ml (sensitivity 78.9%, specificity 62.5%). After Ld therapy, naïve subset of CD4 and CD8 T cells and monocytic 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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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. Paraffin block slices from trephine biopsy material and tumour 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 plasmacytomas 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, Figure1). 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 (n=9) 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 of 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.

Summary/Conclusions: CD56 expression in bone marrow plasma cells significantly increases the OS rate in MM patients regardless the presence or absence of plasmacytomas. 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, L. Simeone1, O. Vitagliano1, S. Palmieri2, S. Rocco2, F. Ferrara2, F. Pane1, L. 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: To have 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 (range 33-81), received BVD (Bendamustine 90 mg/sqm days 1,2,4,5,8,9,11, Dexamethasone 20 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1,4,8,11, Dexamethasone 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression or death. Median time to treatment was 4 months (range 1-12). 22 patients had undergone at least to a single autologous or allogenic SCT. 21 patients were treated with a second line therapy before BVD, including bortezomib, also in combination with dexamethasone. OS in the group of patients (n=4) with CD56+ in extramedullary MM cells and CD56- in bone marrow cells was 67% which was significantly higher (p=0.04) than that in the group of patients (n=9) 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 – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells – 50%

Table 1. PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL

<table>
<thead>
<tr>
<th>Previous regimens</th>
<th>median (range)</th>
<th>median OS</th>
<th>12.5/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>10</td>
<td>6 (2-11)</td>
<td></td>
</tr>
<tr>
<td>del1q</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>73.4% in patients without EMD</td>
<td>56%</td>
<td>56/25</td>
<td></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 permeability via the expression of the endothelial cadherin (VE-Cadherin). Table 1 shows the expression of VE-cadherin in multiple myeloma and may be useful in prognosis. However, the predictive role of VE-cadherin as a prognostic factor for survival of patients after treatment of multiple myeloma is not still clear.

Methods: This study aimed to evaluate the prognostic value of circulating VE-cadherin for progression-free survival in patients with multiple myeloma in complete or partial remission.

Results: Evaluation of the obtained data showed that ve-Cadherin expression was significantly lower in patients with aggressive histology (p<0.05). The expression of VE-cadherin was also significantly lower in patients with EMD (p<0.05). The expression of VE-cadherin was also significantly lower in patients with EMD (p<0.05). The expression of VE-cadherin was also significantly lower in patients with EMD (p<0.05).

Table 1. VE-CADHERIN IN MULTIPLE MYELOMA: AN INDEPENDENT PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL

<table>
<thead>
<tr>
<th>Patient age, years</th>
<th>73.4% in patients without EMD</th>
<th>56%</th>
<th>56/25</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>56/25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: VE-Cadherin is cell adhesion molecules localized at the endothelial junction, which plays critical roles in angiogenesis, neovascularization, neoplasm development, stem cells mobbing, and endothelial integrity. Indeed, VE-cadherin plays a role in the opening and closing of the endothelial barrier. It can be found in 12-14 cases of patients with endothelial dysfunction, as a transmembrane protein that modulates intensity of angiogenesis in multiple myeloma and may be useful in prognosis. However, the predictive role of VE-cadherin as a prognostic factor for survival of patients after treatment of multiple myeloma is not still clear.

Methods: This study aimed to evaluate the prognostic value of circulating VE-cadherin for progression-free survival in patients with multiple myeloma in complete or partial remission.

Methods: One hundred twelve out subjects with multiple myeloma were
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 anthracyclines was used according 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 1.31 ng/ml, with AUC value 0.839 (p=0.0001), the sensitivity and specificity 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 (IC=18.16-11.71) months vs 7.35 (IC=5.75-9.85) 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 gammopathies (MG) is often uninformative, due to 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 – 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), amp(1q21), t(4;14) and del(17p13.1) (TP53 gene) and, starting in April 2016, t(14;16). We had previously established 20 000 cells per sample as the minimum (and sufficient) number of cells needed to get 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 motives underlying the selection of fewer than all 5 probes in samples with a sufficient number (>20 000) of cells included the individual decision of the assisting physician and, for t(14;16), the date of the study. Considering only those studies performed after the introduction of t(14;16), all 5 probes were used in 67.7% of patients; we were able to apply four or more probes in 80% of patients with 1% or less bone marrow PC according to flow cytometry. 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 the 98 patients with a FISH result was 63.6 years old (37.8 to 87.3), and 67.5% of patients; we were able to apply four or more probes in 80% of patients.

**Summary/Conclusions:** We have found that the application of FISH probes in FACS-separated PC is highly efficient with a robust yield, providing a large enough sample for the application of at least two probes in over 95% of patients, irrespective of bone marrow plasmacytosis; in fact, we obtained an average of 165 000 pure PC per patient, which is more than 8-fold higher than the number we consider invariably sufficient to apply 5 probes, which we achieved in at least 80% of patients.

**PB2004**

**CLINICAL SPECTRUM AND EVOLUTION OF MONOCLONAL GAMMAPATHY ASSOCIATED NEUROPATHY VERSUS CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY PATIENTS**


1Seoul Metropolitan Government Seoul National University Boramae Medical Center, 2Seoul National University Hospital, 3Korea University Anam Hospital, Seoul, 4Inje University Haeundae Paik Hospital, Busan, 5Incheon Medical Center, Incheon, Korea, Republic Of

**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, electrodiagnostic studies, and laboratory test results, were enrolled.

**Results:** In both groups, males were predominant. IgG MG was most common (58.8%) 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% versus 91.2%, P=0.001) and ataxia (44.4% versus 81.8%, 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 dysesthesia, pain and sensory symptoms. During median follow-up of 49 months, 2 PPN patients developed overt hematologic malignancies: 1 case of Waldenstrom’s macroglobulinemia and 1 case of AL amyloidosis. Both of them showed malignant transformation within 8 months of neuropathy development, and were associated with worsening neuropathic symptoms. 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 sensorineural 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, progress-free survival (PFS), overall survival (OS). These facts determine individual approach to the treatment and offer rare PFS and cure at various stages of treatment.

Aims: Finding molecular genetic criteria of predicting the effectiveness of autologous peripheral blood hematopoietic stem cell transplantation (auto-PBHSCT) for improving 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 pathogenic mechanisms of the disease, genetic support of patients is essential. It was determined that the carriage of the allele HLA-DQ1*03:02 in MM patients is associated with a high risk of high-dose chemotherapy resistance (F=2.48, p=0.01; OR=7.8, p=0.029), achieving remission after auto-PBHSCT is associated with a carriage of haplotype HLA-C*06 - HLA-DR1A*01:01 (F=4.87, p=0.028; OR=7.3, p=0.008). Abnormalities of chromosomes 13, 14, 15, 16 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 Spirmann=0.42, p < 0.05), deletion of chromosome 17 (17 patients (27.9%), Ro Spirmann=0.41, p < 0.05), deletion/monosomy of chromosome 13 (15 of 25 patients surveyed, Ro Spirmann=0.33, p < 0.05), the translocation t(4;14) (4 patients (6.6%), Ro Spirmann=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 antimyeloma therapy (induction, high-dose therapy (HDT) with autologous stem cell transplantation (ASCIT), 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 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.

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 HDT and ASCT between 2007 and 2016 in a single institution. All patients received high dose stem cell support after conditioning with high dose melphalan (200 mg/m² and 140 mg/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 groups was performed using the log-rank test. The prognostic factors of survival were analyzed by Cox regression univariate and multivariate analysis.

Results: We included 194 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%). 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
Aims: The incidence of leptomeningeal infiltration is unknown.

Background: Leptomeningeal infiltration in patients diagnosed with PCL is a rare and aggressive plasma cell leukaemia (PCL) which is a rare and aggressive plasma cell leukaemia (PCL) [1]. The median age at diagnosis was 57 years (range 35-81) and 8 (47.1%) were males. Clinical and analytical features at the moment of diagnosis are recorded in Table 1.

Results: Seventeen patients with PCL were included. Six (35.3%) were pPCL (≥VGPR) and 9 (52.9%) were sPCL. Median age at diagnosis was 57 years (range 35-81) and 8 (47.1%) were males. Clinical and analytical features at the moment of diagnosis are recorded in Table 1.

Summary/Conclusions: These findings provide evidence for quality of life and PFS after ASCT in patients with MM. Outcome of 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.

PB2009

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 leukaemia (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 of 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 Myeloma Task Force, by the presence of ≥2x10⁹ L peripheral blood PC or plasmacytosis accounting for more than 20% of the differential white cell count. Medical records were retrospectively reviewed. Clinical response was evaluated per IWGM criteria. Clinical and biological features, progression free survival (PFS) and overall survival (OS) were analyzed. Survival curve were estimated using the Kaplan-Meier method and compared using the Log-Rank test.

Results: Seventeen patients with PCL 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.

Table 1.

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 PR in the remaining one. Three patients received intrathecal treatment. The intention-to-treat response was: 2 (15.4%) CR, 2 PR, 7 (53.8%) 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% 0.47-4.7) months and median of OS was 4 (IC 95% 0.47-7.53) months.

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, metotrektate and dexamethasone is not today a standard of care for patients with PCL.
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 (64%) 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 subocclusion, 1 mucositis grade III). 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 by single shot of medium dose melphalan (60 mg/m2) between October 2010 and January 2016.

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 (CiCr), 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 is mainly cleared by glomerular filtration and active tubular secretion in 3 to 4 hours. Serum half-life increases up to 9 hours if moderate/severe renal impairment is present (creatinine clearance <50 or <30 mL/min, respectively). In the latter cases a reduction of the daily dose is recommended. Dose adjustment based on RI severity 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 prolonging the time of full standard doses can be equally effective and tolerated by patients requiring 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 CiCr 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 CiCr 51.4 ml/min, range 20-148). Median progression free survival (PFS) was 10 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.

PB2012

A FEASIBILITY-STUDY ON IMPLEMENTATION OF THE INTERNATIONAL MYELOMA WORKING GROUP RECOMMENDATIONS FOR MULTIPLE MYELOMA PATIENTS IN ROUTINE CLINICAL PRACTICE: A PERIPHERAL CENTER EXPERIENCE

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Background: Renal impairment (RI), defined as serum creatinine above upper normal limit or >2 mg/dl or a estimated glomerular filtration rate (eGFR) <60 ml/min/1,73m2, is one of the most common complications of MM, and it is associated with an increased risk of early death. The incidence of RI at MM diagnosis ranges from 20% to 50%, while its comparison occurred in 60% MM patients (pts). In this scenario tempestive diagnosis of RI in MM pts and exclusion of possible alternative causes of RI (like amyloidosis, diabetes or MIDD) are essential.

Aims: We applied a diagnostic algorithm obtained from the International Myeloma Working Group recommendations in pts admitted to our department for RI (with known and unknown MM, or suspected cast nephropathy, CN), in order to investigate if this diagnostic workflow could positively impact on MM pt management.

Methods: We enrolled adult pts, known or unknown MM, admitted to our hospital for RI or suspected CI, with or without anemia. Primary, we performed complete blood analysis, with eGFR (CKD-EPI and MDRD methods), serum and urine electrolytes, bicarbonatemia, serum and urine immunofixation, fraction 3 and 4 of complement, cryoglobulinemia, HBa1c, arterial gas analysis, evaluation of urine rate every 6 hours, daily urine collection, urine sediment. We also collected anamnesis on eventual nephrotoxic concomitant therapies like ASA, FANS, clinical parameters and objectives signs of RI (edema, symptomatic disionia). On the second day of hospitalization we requested protein electrophoresis on serum and urine, chest X-ray, ultrasonography of abdomen, ecocardiography and electrocardiography. On the day three we evaluated registry of previous exams and we decided, if necessary, to perform surgery or a biopsy (bone marrow in suspected unknown MM pts, renal in suspected CN pts, umbilical fat for amyloidosis). All analyses were daily and colloquially discussed between Internists and Nephrologists.
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 workout 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 dyaalysis and steroids overtreatment.

PB2013
NOCARDIOSIS PROVOKED BY NOVEL AGENTS AT RELAPSED MULTIPLE MYELOMA: CASE SERIES
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Background: The proteosome 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 nocardial 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 bortezomibe 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 1290/mm3 and flow-cytometric analysis showed CD5: %88 and CD20: %1. HRCT showed a 7x6x6 cm sized mass like lesion. Broncoscopic lavage showed brancial 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 after ten days and it showed regression of the infiltration. He was discharged with oral TMP/SMX antibiotic treatment. 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 ponazolimide treatment. Her lymphocyte count was 2300/mm3 and flow-cytometric analysis showed CD5: %98 and CD 20: %1. HRCT showed a 7x6x6 cm sized mass like lesion with a cavity. Branched Gram positive bacillus (Nocardia sp.) was detected from broncoscopic specimen analysis, so imipenem-cilastatin therapy has been started. She responded well to therapy and was discharged with TMP/SMX antibiotic treatment. Case-3: 72 year old man, who has a diagnosis of IgG kappa type myeloma and a history of autologous SCT 4 years ago following bortezomibe treatment, relapsed 5 months ago. He has been admitted to the hospital with non-productive cough under 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 control CT was performed and showed regression. He was discharged with oral TMP/SMX antibiotic treatment.

Results: See Table 1 and Figure 1.

Table 1.

<table>
<thead>
<tr>
<th>General</th>
<th>Case-1</th>
<th>Case-2</th>
<th>Case-3</th>
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</tr>
<tr>
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<td>Male</td>
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<td>CKD</td>
<td>CKD</td>
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<tr>
<td>Present Index</td>
<td>Nocardia</td>
<td>Nocardia</td>
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</table>

Summary/Conclusions: The proteosome 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 naïve multiple myeloma (MM), Sqr-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 >221μmol/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 alternately well-cited pharmacological dosing that the more appropriate starting dose is likely 15-20mg three times per week, 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. Patients’ baseline characteristics, prior therapies, cytogenetics and FISH data were collected.

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 3 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</th>
<th>Gender</th>
<th>Renal Function</th>
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<th>Present Index</th>
<th>Present Index</th>
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<tr>
<td>72</td>
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<td>Lenalidomide</td>
<td>Nocardia</td>
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</tr>
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</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 were on dialysis only. There was significant variation of dose-related tolerability between patients. However, toxicity was manageable with diligent monitoring and dose adjustment.
RESULTS:

Data was analysed and interpreted via IBM SPSS Statistics version 24.0. The relevant information was entered into a pre-designed data collection form.

Aims:

Methods:

AIMS:

To establish treatment and outcome of patients with multiple myeloma; To establish treatment and outcome of patients with multiple myeloma (serving a population about 1 million people). It is 980 km away from its main of Borneo. Sarawak General Hospital is the tertiary referral center of Sarawak Malaysia.

EXPERIENCE

biochemical response.

to follow up patients after the first line treatment added to the evaluation of the biochemical response obtained after treatment was positive in patients with complete response. Twopatients had PET/CT with progression disease and corresponded to a biochemical progression.

response was defined according to the standard IMWG response criteria.

PET/CT.

Aims:

help to promptly define response or failure to the treatment.

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 second FDF PET/CT.

Methods:

We included in this retrospective and observational study at University Hospital of 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) with 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 immunorepress. 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, MPV, and LD. 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 the correlation between PET/CT and biochemical response. The results obtained were traced and the relevant information was entered into a pre-designed data collection form.

Data was analysed and interpreted via IBM SPSS Statistics version 24.0.

Results:

There were a total of 63 patients with the male to female ratio of 3:2. The median age for patient was 61 years old (range 31 to 86 years old). Majority of them were local natives of iban or Bidayuh descendants (n=32, 50.8%) followed by Chinese (n=20, 31.7%) and Malays (n=11, 17.5%). Most common type of multiple myeloma is of IgG variant (n=27, 42.9%). The most common myeloma related organ or tissue impairment (ROTI) are anaemia (n=54, 85.7%) followed by bone lesion (n=48, 77.8%), renal impairment (n=27, 42.9%) and hypercalcemia (n=18, 28.6%). More than half presented late with Durie Salmon stage III disease (n=34, 54%). Majority of patients were treated with dexamethasone/thalidomide (n=25, 39.7%). Sixteen patients (25%) received bortezomib based treatment. Three patients (n=3, 4.8%) undergone bone marrow transplant. Thirty five patients died (n=35, 55.6%). Median survival time was 21 months (95% CI: 16.26). One year, two years and five years survival rate was 67.4%, 43.6%, 31.6%. Patients who were 60 years old and above have lower median overall survival (20 months) compare to patients who were 60 years and below (36 months) even though they are not statistically significant (p=0.565).

Summary/Conclusions:

Baseline characteristics of patients with multiple myeloma in Borneo Sarawak are similar to the rest of Asia. However, our median an overall survival was comparatively lower to our counterparts. Limitation wise, due to logistic and economic reasons, we do not have good access to cytogentic and genetic profiling that enables us to prognosticate patients accordingly.

PB2017

A RETROSPECTIVE AND PROSPECTIVE AUDIT OF RADIOLoGICAL INVESTIGATIONS FOR SUSPECTED CASES OF PLASMA CELLo MYELOMA IN THE ALTNAgELVIN 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 myeloma 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 for myeloma to assess for bone disease or EM plasmacytoma.

Methods:

This retrospective and prospective audit included all patients having a skeletal survey performed for suspected multiple myeloma within the Alt-nagelvin Area Hospital (AAH). Retrospectively from 10/2/15 until 9/2/16 data was collected using the advanced search feature of the Sectra 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 pre guidance.

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- ed 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 to 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 1. 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 IIIB (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 3cases, 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 double: - Symptomatic: alkaline hydration, correction of the metabolic disorders and sometimes the renal extra purge (indicated in 3cases). - Specific: chemotherapies VAD 7cases, 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 achievement 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 reducing the excretion renal of the light chains.

PB2019

DEPP RESPONSES WITH CARFLIZOMIB-LENALIDOMIDE-
DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: A REAL LIFE EXPERIENCE
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Background: Carfilzomib is a new proteasome inibitor 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 lenalidomide and dexamethasone. In the phase I dose-escalation part the maximum plato 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 explorer 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, and 6 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 “Cardinale 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 12-82); 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; 2 (14%) prior therapy with pomalidomide (Table 1). Eleven (73%) patients achieved after 2 cycles a response rate 2PR, 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>62 (18-79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MULTIPLE MYELOMA, n (%)</td>
<td></td>
</tr>
<tr>
<td>RELAPSED</td>
<td>11 (60)</td>
</tr>
<tr>
<td>REFRACTORY</td>
<td>5 (34)</td>
</tr>
<tr>
<td>MULTIPLE MYELOMA SUBGROUP, n (%)</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>4 (40)</td>
</tr>
<tr>
<td>IgA</td>
<td>2 (20)</td>
</tr>
<tr>
<td>MICROMOLECULAR</td>
<td></td>
</tr>
<tr>
<td>STAGING, (%)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2 (20)</td>
</tr>
<tr>
<td>II</td>
<td>12 (80)</td>
</tr>
<tr>
<td>III</td>
<td>5 (40)</td>
</tr>
<tr>
<td>IV</td>
<td>8 (53)</td>
</tr>
<tr>
<td>MEDIAN TIME FROM DIAGNOSIS TO KR, months (range)</td>
<td>46 (12-92)</td>
</tr>
<tr>
<td>MEDIAN OF PRIOR THERAPY, lines (range)</td>
<td>3 (1-4)</td>
</tr>
<tr>
<td>PRIOR TRAPLANT, n (%)</td>
<td>9 (60)</td>
</tr>
<tr>
<td>AUTOLOGUES</td>
<td></td>
</tr>
<tr>
<td>ALLOGENEIC</td>
<td>1 (6)</td>
</tr>
<tr>
<td>PRIOR THERAPY, n (%)</td>
<td></td>
</tr>
<tr>
<td>LENALIDOMIDE</td>
<td>11 (73)</td>
</tr>
<tr>
<td>BORTEZOMIB</td>
<td>15 (100)</td>
</tr>
<tr>
<td>POMALIDOMIDE</td>
<td>3 (14)</td>
</tr>
</tbody>
</table>

Figure 1.
Background: Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective in relapsed and refractory Multiple Myeloma (rrMM), whose prognosis is particularly severe.

Methods: 21 patients (12 M/9 F, Table 1), with rrMM, median age at diagnosis 62 years (r. 47-75), median age at start of treatment 65 years (r. 53-81) treated with several lines of treatments (median 3, r. 2-10), included 2 patients refractory to Bortezomib, underwent to KRD regimen (ASPIRE trial schedule: Carfilzomib starting dose 20 mg/sqm on days 1,2 of cycle 1, target dose 27 mg/sqm thereafter; Lenalidomide 25 mg on days 1 through 21; Dexamethasone 40 mg on days 1,8,15 and 22, every 28 days) for a median treatment cycles of 2 (r. 1-6) with 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 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.

Results: Carfilzomib 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 hospitalization was required, no septic shocks were observed); 33% grade 2, 19% grade 3 and 5% grade 4 thrombocytopenia, without hemorrhagic events and necessity of transfusions. Concerning severe extrahematologic toxicity, it was observed grade 1 pneumonia in 47% of patients, due to 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% (14/21 : 8 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 rrMM 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.

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.

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(IMWG’03) established for 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) attributable 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 Plasma myeloma) 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 multi-tropic involvement. 7 of these NDMM were smoldering MM (sMM). All of them then completed initial staging with more sensitive imaging tests than conventional radiology (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 criteria and the last one with BM Plasmatic Cell (BMPC) >60%, MRI image and FLC criteria.

Summary/Conclusions: Of the hypotheses for introducing new criteria for initiating treatment was that the initiation of adequate available early treatment may improve the prognosis of patients with symptomatic NDMM. In an aging population such as the one we present, we believe that these new criteria to initiate treatment can improve the medium- and long-term prognosis of this group of people with few chance to start intensive or a lot of lines of treatment because of increasingly comorbidities by age. Further follow-up and evaluation of survival comparing the “classical” group vs new-criteria group are guaranteed to assess if these early treatment will improve survival.
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 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 unacceptably 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: 56.0-80.25), 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% (only 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 6 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 events, 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 single nucleotide polymorphism (c.1621 A>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 specifically acquired in immature response to PDGFRαβ abnormalities (Jorfo 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 (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 control and HE groups. Digital droplet PCR (ddPCR) was used for patients heterozygous for KIT M541L by the ARMS assay 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; 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 PREDICTIVE OF PROGRESSION TO HEMATOLOGICAL NEOPLASMS IN PATIENTS WITH IDIOPATHIC LEUKOCYTOPSY

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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, SF3B1, and JAK2 V617F and ETNK1 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.

Methods: We previously analyzed the JAK2, CSF3R, CALR, SETBP1, and ETNK1 genes in 10 patients with idiopathic leukocytosis (EH20). To elucidate the relevance of genetic alterations, we extended the analysis with 17 genes known to be involved in hematological neoplasms in 16 patients with idiopathic leukocytosis.

Results: Leukocytosis is defined as a total white blood cell count more than two standard deviations above the mean, or a value greater than 11,000/μL.

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.
of time. The period of observation was 1 year or longer in most patients. Sixteen patients with idiopathic leucocytosis 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 and/or and/or antibody specific polymerase chain reaction analysis. Total RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) were performed between the ETV6 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 leucocytosis. No mutations were found in the other genes in the 16 idiopathic leucocytosis patients. ETV6-ABL1 fusion gene was detected in one of the 10 patients. No BCR/ABL1 fusion gene was detected in the 8 patients. One patient had idiopathic leucocytosis associated with hematopoietic malignancies. The objective of this work is to investigate the presence of specific microRNAs in isolated microvesicles derived from bone marrow aspirates of patients with idiopathic leucocytosis and to determine any potential relationship with the disease. The presence of microvesicles in the bone marrow aspirates of patients with idiopathic leucocytosis suggests a role for microvesicles in the pathogenesis of the disease. The study was conducted in accordance with the guidelines and with the approval of the Ethics Committee.

Summary/Conclusions: Idiopathic leucocytosis comprises heterogeneous conditions. JAK2 mutations and cytogenetic abnormalities are predictive of progression to hematological neoplasms.
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 (c-kit), and platelet-derived growth factor receptor alpha (PDGFRα), have been described in almost all patients with BCR-ABL negative MPNs. JAK2 mutations are present virtually all cases of Polycythemia Vera and 50-66% of prMF and Essential Thrombocythemia (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 immunoreactivity was seen in 8 (25%) of all pr MF patients. CALR immunostaining not seen in patients with PMF myelofibrosis who are negative for JAK2V617F mutation. CALR immunoreactivity was not seen in patients with PMF myelofibrosis who are positive for JAK2V617F mutation. CALR immunoreactivity was seen in 3 (100%) of patients with ET and 2 (100%) of patients with known CALR mutation. CALR immunoreactivity was seen in 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 erytoid 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.

PB2028
THE HIF1A/2A MRNA INDEX HAS A SIMILAR TREND AS THE CHANGES OF EXPRESSION MRNA CALR AND MDR1 GENES IN WHOLE BLOOD SAMPLES OF PATIENTS WITH JAK2 V617F POSITIVE MPN
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Background: Various groups have reported that isofoms of hypoxia-inducible transcription factor 1α (HIF-1α) and 2α (HIF-2α) can regulate both overlapping and distinct target genes. HIF-1α and HIF-2α have been shown to play opposite roles in the regulation of macrophage function [Takeda N. et al., 2010]. HIF-index incorporated as a strong prognostic biomarker of renal cell cancer [Szendrői A. et al., 2016]. Only HIF1α was known as regulator expression of multidrug resistance gene (MDR1) and response to chemotherapy [Comerford K.M. et.al., 2002]. 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 immunoreactivity was seen in 8 (25%) of all pr MF patients. CALR immunostaining not seen in patients with PMF myelofibrosis who are negative for JAK2V617F mutation. CALR immunoreactivity was not seen in patients with PMF myelofibrosis who are positive for JAK2V617F mutation. CALR immunoreactivity was seen in 3 (100%) of patients with ET and 2 (100%) of patients with known CALR mutation. CALR immunoreactive cells were seen in parallel 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 erytoid 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.

CD177 EXPRESSION IN PERIPHERAL BLOOD NEUTROPHILS IN HEALTH AND DISEASE STATES
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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 peripher- al 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. Polychytemia 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, 1.5-25.7; 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 in either PV and ET patients. With a 0.05 cut-off 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 respectively. 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.
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.

PB2030
DETECTION OF THE MUTATIONS IN GENES JAK2 AND MPL IN THE DIAGNOSIS OF CHRONIC MYELOPROLIFERATIVE DISORDERS
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Background: Chronic myeloproliferative diseases is a group of clonal Ph-negative hematological diseases, which include erythremia (polycythemia Vera, PI), chronic megakaryocytic leukemia (essential thrombocytopenia, ET) and subleukemic myelosclerosis (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 thrombocytopenia 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 Interlaborservice, 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 thrombocytopenia in 42 patients of the 78 (54,1%), with chronic idiopathic myelofibrosis in 27 patients of 55 (49,1%). In 67 patients with no hematological profile, was 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 V617FJAK2-negative patients exclusively diagnosed with polycythemia Vera. The MPLV651L 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.

PB2031
ASSOCIATION OF MYELOPROLIFERATIVE NEOPLASM AND LYMPHOPROLIFERATIVE DISORDER IN 3 PATIENTS
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1 Laboratoire, Hopital d’instruction des armées Desgenettes, 2 Laboratoire, Groupement Hospitalier EST, 3 laboratoire, Groupement hospitalier Sud, 4 department of cytogentic and molecular biology, Groupement Hospitalier EST, 5 department of oncolgy and hematology, Centre Leon Barad, 6 laboratoire, Groupement Hospitalier EST, 7 laboratoire, 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 cytogeneric 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, anemia 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- Medullary 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 acetil salicylic acid. 5 years after, she presented with IgG kappa type monoclonal gammopathy up to 28 g/L, without any associated clinical manifestations nor cytopenia. Medullar blood was diluted but showed slightly atypical plasmocytes remaining under 10%.Myeloma was diagnosed anyway and the patient received 5 cures of Velcalde-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 thrombocytocytopenia (platelets: 1886 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)(148/100). 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 MPNLPD 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 pathalogical associations: genomic instability due to JAK2 protein kinase activation, or due to BCR-ABL mutation, or exposure to cytotoxic chemotherapy or radiations (3).
Myeloproliferative neoplasms - Clinical

PB2032

CLINICAL AND ANALYTICAL DIFFERENCES BETWEEN CALR TYPE-1 AND CALR TYPE-2 MUTATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS: A SINGLE CENTER STUDY

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Background: The JAK2V617F is a major 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 mutation was recorded in ET patients without CALR mutations.

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 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 positive 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 type-1 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) and 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. 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 respect to the International Prognostic Score System (IPSS).

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.

PB2034

THROMBOTIC AND BLEEDING RISK FACTORS IN ESSENTIAL THROMBOCYTHEMIA

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1Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

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 (triple-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 were 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 CALR+ – 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 1. Number of line treatementes 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>

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>
</tr>
</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+1) and 837x10^9/l (hemorrhage+) (p=0.003). No significant statistical differences in median hemoglobin and leucocyte count (p=0.75 and p=0.47) were detected. There were more than a half pts older than 60 years in groups NC (51%) and thrombosis+ (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 >1000x10^9/l and leucocytosis >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).

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 N1-5 patient mutations was determined by pyrosequencing: N542-E543del (c.1624_1629delAATGAA); I540-E543delinsKK (c.1619_1627 TCA-gAAATgK (c.1622_1627delGAAATG) and p.H538_K539L (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 N1, 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. N1-4 patients was treated phlebotomy only and did not received any cytoreductive treatment to date. Patient N5 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 N1 patient is significantly increased in the disease dynamics.

Summary/Conclusions: Leukocytosis >11x10^9/l and thrombocytosis >1000x10^9/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.

**Table 1.**

<table>
<thead>
<tr>
<th>pt</th>
<th>JAK2 exon 12 mutation</th>
<th>Thrombosis</th>
<th>Leukocytosis</th>
<th>Hemoglobin</th>
<th>Thrombocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>JAK2V617F</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>JAK2V617F</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>JAK2V617F</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>4</td>
<td>JAK2V617F</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>JAK2V617F</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**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.
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 hybridisation based enrichment technology and consists of 25 MPN-related genes. During the validation stage the following were enriched and analysed: 29 positive control trol 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 I 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 present in 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 NEAPLASMS, 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 hemorrhagic events. Our aim was 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 use were recorded. Hemorrhagic complications were classified as “major” or “minor” in agreement with ISTH criteria. The patients were categorized into four groups based on the amount of JAK2 mutant allele: (1) 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 with 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 higher in the 1st quartile and decreased both to 2nd (p=0.004) and to 1st (p<0.001). The incidence rate of hemorrhages are respectively 0.7/1000 pats /y for 1st quartile, 0.65/1000 pats /y for 2nd quartile, 1.26/1000 pats /y for 3rd quartile and 3.23/1000 pats /y for 4th quartile with a IRR of 5 and 4.6 for the 4th quartile respectively versus 2nd and 1st one. No statistically significant differences were observed 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 an 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 evaluate 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), 56 males with a median age of 65 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. The 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 ERYTHROCYTOSIS 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 2016. 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.
The fusion genes of rearrangements of PDGFRA 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 PDGFRA 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 eighth 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. The rearrangement achieve rapid and durable remissions of IM.

Summary/Conclusions: In summary, patients with significantly anemia and eosinophilia should be screened for the presence of PDGFRA and B rearrangement. 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 PDGFRA and B abnormal was similar with a previous report in a western population and another Chinese hematology center.

PB2040

PLATELET AGGREGATION STUDY OF ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE

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Background: Essential thrombocytosis (ET) is a myeloproliferative neo-plasm 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 600 x 10^9/L, in the recent recommendation, the standard prolongation of light transmission aggregometry by the platelet's 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 cases of MPN, 1 case of AML-M2 and 1 case of non-hodgkin lymphoma. Whole blood aggregometry (WBA) and LTA with 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 900 x 10^9/L, the platelet aggregation problem was not significantly different (ADP-induced PA: p=0.986, collagen-induced PA: p=0.514).

Summary/Conclusions: In the ET patients with platelet counts more than 900 x 10^9/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 neo-plasms 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.

**Method:** 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) (46%). 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 GPT, hypalbuminemia were significantly advanced in disease variant 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 investigation 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.

**PB2045**

**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 (>450x10^9/L) in the blood counts, whose cause can be primary or secondary. JAK2 V617F was described. Hereditary thrombocytosis is a rare congenital disease due to germ line mutations affecting thromboxin 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-hemoragic 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±90x10^9/L; hemoglobin- 13,3±1,2 g/dl and leucocytes- 9,3±1,3x10^9/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 the identification of JAK2 T875N mutation in 3 adults previously characterized as essential thrombocytopenia (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, 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.**
ment recommendations. However, this had no impact on satisfaction suggesting that UK patients welcome an open discussion on treatment options with their physician. These data highlight the importance of maximizing patient physician communication in order to improve patient satisfaction with treatment in the UK.

**PB2046**

**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 thrombocythemia (ET) and primary myelofibrosis (PMF). Driver mutation’s confer growth advantage on the cancer cell and most is 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 driver mutations in patients with 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 RQ-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 has the lowest allele burden, those with PV an intermediate one and those with PMF showed the highest burden. Measurement of JAK2V617F allele burden by RQ-PCR for a PMF case after allogeneic transplant (T.Klampf, 2013) 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, 4 cases with a 14bp 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 49 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%/65% 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/Conclusion:** The effect of type of driver mutation on the clinical and laboratory features of the ET and PMF has been found. Type of CALR mutations in our study had no effect on the number of PLT in ET, but have a value for this index in PMF. Type 1 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 effect of type I 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 and DIPSS.

**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 thrombocythemia (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). There 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 PM.

**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% of 9.6% (p=0.178). Mutations of type I detected in ET, in 18 cases - in PMF, type II in 13 cases - in ET and 7 - 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 (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 - 119.6mm (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 mutations associated 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 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 effect of type I 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.**

**PB2049**

**THE UNIQUE CASE OF GERMLINE CEBPA MUTATION IN PATIENT WITH FIP1L1/PDGFRAS ASSOCIATED MYELOID/LYMPHOID NEOPLASM WITH EOSINOPHILIA**

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**Background:** Myeloid/lymphoid neoplasms with eosinophilia (MLNe) associated with PDGFRA rearrangement are rare disorders. The most frequent PDGFRA abnormalities is FIP1L1/PDGFRA (F/P) fusion gene results from a cryptic interstitial deletion at 4q21 with constitutive activation of tyrosine kinase (TK) activity. Although known since 2003, many questions remain in understanding the biology, disease course and response to therapy. The F/P fusion gene may clinically present as chronic eosinophilic leukemia (CEL), T-cell lymphoblastic lymphoma (T-LL), T-cell lymphoma, chronic eosinophilic leukemia (AML) may also occur at presentation or during the course of the disease. While F/P is the driver mutation, to date there are few data about genetic variants of the disease that may contribute to clinical outcome. CCAAT/enhancer binding protein alpha (CEBPA) gene functions as key regulator of granulocytic differentiation, CEBPA mutations contribute to leukemogenesis by promoting proliferation and blocking differentiation of myeloid lineage in AML. Germline CEBPA mutations is a very rare and account about 1% in AML only.

**Aims:** We present the first case of detection of familial germline CEBPA muta-
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 (20x10⁹/L), with marked eosinophilia (4.0x10⁹/L). A bone marrow aspirate showed 2% blasts, 21% eosinophils. Cytogenetic analysis of an unclotted lymph node biopsy suggested diffuse proliferation of medium-sized lymphoblasts. Immunohistochemistry and flow cytometry showed that the lymphoblastic population expressed CD2, CD5, CD7, CD4, CD99, TdT and CD1a. Polymerase chain reaction (PCR) analysis from samples of the lymph node and bone marrow failed to detect N-terminal T-cell 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), bone marrow cellularity and stem cells have been successfully harvested after stimulation of hematopoiesis. However, within 10 days after the discontinuation of G-CSF he developed leukocytosis (130x10⁹/L) with 21% of eosinophils (absolute number 27.3x10⁹/L) and cibulal 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 4q12 (F/P rearrangement), confirmed by RT-PCR. The same N-terminal (TAD2) CEBPA mutation was found in the brother’s skin, bone and marrow, and in the patient’s brother bone marrow samples. Unfortunately, no materials from parents was available for analysis at that time.

Results: The same N-terminal (TAD2) CEBPA mutation was found in the patient’s skin, bone and 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 are 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 patient with PFDGRA-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 NEPLASMS PATIENTS

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Background: 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 Surveyed World (ROSW).

Methods: A cross-sectional survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK. The internet-based survey was administrated separately to MPN patients and treating physicians (the two samples were not linked). Observed differences between the UK and ROSW are described in terms of symptom burden.

Results: A total of 699 pts (UK, n=286; ROSW, n=413) and 219 physicians participated in the survey. UK patients reported more symptoms than those in ROSW (9.02 vs 5.95 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; ROSW - 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%; ROSW – 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 ROSW for the three most common symptoms: fatigue and tiredness (mean: UK 6.73, ROSW 5.67; PV, mean: UK 6.91, ROSW 5.95; ET, mean: UK 7.10, ROSW 6.57). This difference was not observed when physicians were asked to rate symptom severity. An overall symptom burden was calculated as a function of all patient-reported symptoms. UK patients were disproportionately represented in the high symptom burden group (22% vs ROSW 16%) if compared to the 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 current treatment of AML compared to conference attendees; 81% satisfied with treatment (UK 81% satisfied vs ROSW 90%) and disease management (UK 87%, ROSW 90%). However, 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 26.25; ROSW – 15.31). ROSW patients were also more likely to agree with the statement ‘There is not enough time during the appointment to discuss all of the symptoms a patient is experiencing’ (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 counterparts, but are also more likely to feel 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.

PB2053

FINAL RESULTS FROM PEN-PV STUDY, A SINGLE-ARM PHASE 3 TRIAL ASSESSING THE EASE OF SELF-ADMINISTERING ROPEGINTERFERON ALFA-2B USING A PRE-FILLED PEN IN POLYCYTHEMIA VERA PATIENTS


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Background: Interferon-alpha (IFNa) based therapies have been successfully used in myeloproliferative neoplasms for over thirty years. A known burden for long-term therapy applying IFNa in otherwise fit outpatients is the necessity of frequent hospital visits for product administration. Rogepinterferon alfa-2b (AOP2014) is a novel long-acting monopegylated IFNa allowing initial bi-weekly and, in long-term maintenance, monthly administration. To further improve on convenience and compliance, a pre-filled, dose-adjustable pen was developed for patient self-administration at home.


Methods: The study was performed in 18 sites in 8 European countries. Patients were eligible who completed the AOP2014-arm in the PROUD-PV study (12 months of treatment). A total of 7 visits was scheduled within 3 months (two supervised self-administrations at site, followed by self-administrations in the home-setting, and one final assessment visit at study site).

Results: A total of 36 patients were enrolled and received the AOP2014 pen for self-administration. The mean age was 58.5 years (range 37 to 77 years), 23/36 (63.9%) were male patients and a large proportion of patients (28/36) had a history of previous treatment. The patients were treated for a median duration of 21 months (range 6 to 41 months). The overall compliance was 92% (n=33). The reasons for non-compliance included: symptoms of the disease, side effects, cost of the medication, not being able to remember to take the medication, transportation issues, and fear of the medication.

Summary/Conclusions: MPN10 score is directly affected by JAK2 and CALR positivity and can be used as a major predictor of survival in MPNs 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.
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.

PB2054

JAK2, CALR AND MPL MUTATIONS: CORRELATION WITH PHENOTYPE DISEASE AND HISTOPATHOLOGICAL FEATURES OF BONE BIOPSY

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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 phenotypic 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 evaluated 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 were present 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 patients are show 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 laboratory features of the patients are show 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 megalacaryocytes according with the mutational status and there were more frequent in patients with CALR 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>Genome</th>
<th>Subgroup</th>
<th>Initial JAK2 V617F allele (%)</th>
<th>Follow-up JAK2 V617F allele (%)</th>
<th>Difference</th>
<th>Initial CIC</th>
<th>Follow-up CIC</th>
<th>Treatment</th>
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<tr>
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</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 megalacaryocytes 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.

PB2055

CLINICAL IMPLICATION OF QUANTITATIVE JAK2 V617F ANALYSIS WITH DROPLET DIGITAL PCR IN MYELOPROLIFERATIVE NEOPLASMS

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Background: JAK2 V617F is the most common genetic mutation in myeloproliferative neoplasms (MPN) and included in the major diagnostic criteria. Beyond the description of existence, quantification of mutational load is proposed as a useful information to classify subgroups of MPN and to predict prognosis. Droplet digital PCR (ddPCR) is a novel assay which has an advantage in accurate and reproducible quantitative analysis.

Aims: This study was planned to verify the correlation of ddPCR with pyrosequencing in diagnosis of MPN and to investigate clinical implication of the mutational burden in disease course.

Methods: Between 2012 and 2016, peripheral blood or bone marrow samples were obtained from 56 patients at diagnosis and every 3 months after enrollment. Inclusion criteria were 1) older than 20 years, 2) who were newly diagnosed with MPN and 3) diagnosed with MPN before, not met the indication of JAK2 inhibitor treatment yet. JAK2 V617F mutation was detected by pyrosequencing as diagnostic work-up. The ddPCR was performed using the same samples with pyrosequencing to prove correlations between assays and to establish a detection sensitivity cutoff. Clinical aspects and hematologic profiles of enrolled patients were reviewed.

Results: The lowest value of measured JAK2 V617F allele by ddPCR except negative samples in our study was 0.01%, which was approximately 0.07 copies/μL of mutant allele. Some discrepancies were observed from 0.0001% to 0.01% concentration between the expected and measured values in ddPCR detection sensitivity assay. 0.1% was determined as the cutoff. Forty-two patients (75%) were positive for JAK2 V617F by pyrosequencing and 46 (82.1%) were positive by ddPCR. The mean mutated allele at diagnosis was 37.5% ±30.08%. With ddPCR, the mean was 40.7% ±31.2%. Pyrosequencing and ddPCR were highly correlated (r=0.9712, P<0.001). JAK2 V617F burden measured with ddPCR was significantly different by subgroups (P<0.001). In comparison of one disorder with another, polycythemia vera (PV) had more amount of mutant allele than essential thrombocythemia (ET) (P=0.001), however, differences between PV-myelofibrosis (MF) and ET-MF were not statistically significant. Follow-up samples were available in 12 patients and 8 were JAK2 V617F positive. Among them, reduction of mutant burden after treatment was observed in 6 patients (75%). JAK2 V617F burden showed initial reduction in a MF patient treated with JAK2 inhibitor, however, after dose reduction for toxicities, the JAK2 V617F mutation increment with hematologic aggravation was discovered. Mutation burden decreased showed a tendency consistent with hematologic improvement.

Hematologic characteristics and JAK2 V617F load at the initial diagnosis and follow-up after treatment (Table 1, Figure 1).

Table 1.

<table>
<thead>
<tr>
<th>Pl. Nr.</th>
<th>Genome</th>
<th>Subgroup</th>
<th>Initial JAK2 V617F allele (%)</th>
<th>Follow-up JAK2 V617F allele (%)</th>
<th>Difference</th>
<th>Initial CIC</th>
<th>Follow-up CIC</th>
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<tr>
<td>M02</td>
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<td>ET</td>
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</tr>
</tbody>
</table>

Figure 1.
Clinical Impact of Jak2 and Carletticulin Gene Mutations in Patients with Essential Thrombocythemia

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Background: Jak2 (V617F) gene mutation is found in approximately 60% of patients with Essential Thrombocythemia (ET), while 5-10% of Jak2 (V617F) negative ET patients carry MPL gene mutations including 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+), 12 (8.1%) carried a mutation at exon 9 of CALR gene (CALR+), 3 (2.1%) a mutation at codon 515 of MPL gene, 26 (17.5%) patients were not mutated for Jak2, CALR and MPL genes (triple negative). Jak2+ subjects, compared to Jak2− patients, had a younger age at diagnosis: median age 43.5 years (22-92) in Jak2+ patients vs 72 years (18-93, respectively). Patients with MPL mutation had a median age of 82 years while triple negative patients 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 Thrombocythemia (IPSET) categories was also statistically significantly different (p=0.003) for the three groups. The percentage of high-risk patients was 0% in Jak2+• MPL+ group, 19% in Jak2+• CALR+ group, and 21% (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(32/97) 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, 30% (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: Jak2+ 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 categories in CALR+ patients. The prevalence of CALR mutations suggests a second hit in the multistep pathogenesis of ET.

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 and constitutional symptoms in adults with primary myelofibrosis (PMF), post-essential thrombocythaemia and post-polycythaemia vera myelofibrosis (PPV-MF), and post-essential thrombocythaemia myelofibrosis (PET-MF).

Aims: We present a retrospective multicentre analysis of MF patients treated with ruxolitinib from August 2012 to December 2016 at 3 centres in the East of England to assess its efficacy, safety, and tolerability in a ‘real-world’ clinical setting.

Methods: Retrospective data collection using electronic medical records and cancer registry data identified 49 MF patients treated with ruxolitinib at the James Paget, Norfolk and Norwich, and Ipswich hospitals (28, 14 and 7, respectively) over a 52-month period. Five had less than 3 months follow-up and were excluded.

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 (unclassifiable)-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 7 (15.6%) patients. 3 weeks on treatment was most common (17/46) followed by intermittent therapy (11/46) 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/Foxon 12-unmutated, and 1 (3.1%) was CALR-mutated. The median duration of treatment was 16.4 months (3–45). We recorded 25 infectious events at diagnosis of ET was 0 in the CALR+ group, 28, 03% (30/107) in the JAK2+ group, and 18, 30% (18/107) in triple negative group. This was statistically significantly different (p=0.001) among the three groups: the incidence of thrombotic events at diagnosis of ET was 0 in the CALR+ group, 28, 30% (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: Ruxolitinib was well-tolerated and effective in improving constitutional symptoms in our ‘real-world’ study population. Therapeutic response and safety profile was similar to trial data although we observed a higher incidence of minor haematologic AEs that were readily managed with supportive care. Weight gain was associated with a strong survival advantage and could prove a useful clinical marker of response. The majority of patients remain on active treatment.

Monitoring of Transient Myeloproliferative Disorder and Leukemia in Down’s Syndrome: A Single University Hospital Study

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1Pediatrics, AJOU University School of Medicine, Suwon, Korea, Republic Of

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. These patients has leukocytosis, and in 18 case found blast cells in their peripheral blood. These patients has leukocytosis, and in 18 case found blast cells in their peripheral blood. These patients don’t suffer leukemia and TMD, to determine prognosis and risk factors.

Aims: This study gathered DS patients to find some difference between leukemia and TMD, to determine prognosis and risk factors.

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 AML, 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 but didn’t confirm former examination. 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, 4 AML. All AML patients had additional chromosomal change 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.
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 (IL-1, IL-6 and TNFalfa) 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 cytoeducive 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 was 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 these infections were treatable by appropriate supportive care.

Summary/Conclusions: These data in our small series of patients suggest a higher incidence of ruxolitinib associated infections observed in clinical practice compared to traditional treatment. Immunosuppressive effect of Ruxolitinib is reported and the use of this drug in the transplant setting with beneficial effects on alloreactivity and on graft versus host disease is becoming more common. These patients might benefit from receiving prophylactic therapy with antiviral drugs or antibiotics or antifungal therapy or in alternative by careful monitoring. Finally nowadays physicians and patients should be aware of potential risks of using ruxolitinib including the risk of infections. In summary, infections can occur in patients treated with ruxolitinib but are generally mild. Generally infections were non-life threatening and managed with appropriate supportive care. Special care probably should be taken for patients older (more than 75 years old), treated with corticosteroid and with renal impairment. However larger studies are needed to confirm these observations.

PB2060

THE JAK2V617F MUTATION AND LEUKOCYTOSIS AS RISK FACTORS FOR INCIDENCE OF THROMBOTIC COMPLICATIONS IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background: Polycythemia vera (PV) is a chronic, clonal, progressive myeloproliferative disease, caused by transformation of pluripotent hematopoietic stem cell. It is a malignant hematological disease that leads to excessive proliferation of erythroid, myeloid and megakaryocytic elements in the bone marrow. Essential thrombocythemia (ET) is a clonal disorder of unknown etiology characterized by increased numbers of multipotent hematopoietic stem cell, and it is characterized by enhanced formation of megakaryocytes in the bone marrow and for no apparent cause, by markedly increased platelet counts in peripheral blood. PV and ET belong to a group of Philadelphia chromosome negative myeloproliferative neoplasms. Thrombotic and hemorrhagic complications are the most common causes of morbidity and mortality in patients with PV and ET. It is thought that the mechanisms that lead to thrombosis in MPN are the following: increased blood cell mass, abnormal platelet function and the phenomenon of spontaneous aggregation. The contribution to the incidence of thrombosis: increased level of products that are formed in the activation of platelets (thromboxane, p-selectin); increased production of microparticles that are parts of various cell membrane structures of platelet origin; JAK2V617F mutation. In patients with MPN there is increased activity of the coagulation system due to the resistance to the anticoagulant function of thrombomodulin.

Aims: The aim of this study is to monitor JAK2V617F mutations and leukocytoses as potential risk factors for the development of thrombotic complications in patients with polycthemia vera and essential thrombocythemia.

Methods: During the five-year period we monitored the occurrence of thrombotic complications in 56 patients (of both sexes, aged between 30 and 78 years), being diagnosed with PV and 22 patients (of both sexes, aged between 38 and 79 years) being diagnosed with ET. We used methods of clinical, laboratory, ultrasound and CT scans. With regard to the risk factors we followed the presence of JAK2V617F mutations and leukocytoses.

Results: Leucocyte count ranged from 5,2-27,1 x 109/L. The highest leucocyte counts recorded in the group of patients with PV (p>0,01). JAK2V617F mutation was also statistically more significantly present in patients with PV. The highest percentage of thrombotic complications (arterial and venous) was found in the group of patients with ET, which was statistically more significant relative to PV. Thrombotic complications in those groups were more frequent in patients diagnosed with leucocytosis, but statistical significance was present only in the group with PV. Thrombotic complications were more frequent in both groups with thrombosis in percentage with JAK2V617F positive patients, but without statistical significance. It is believed that activated neutrophils bind to platelets by influencing the increased expression of tissue factor activity, as well as the activation and damage of the endothelial cells, especially with JAK2V617F positive patients.

Summary/Conclusions: Leukocytosis and JAK2V617F may be considered as potential risk factors for the incidence of thrombosis in patients with PV and ET. Further follow-up of those patients, as well as a larger number of subjects are needed.

PB2061

RISK FACTORS FOR INCIDENCE OF HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN) are the group of clonal, malignant hematopoietic stem cell disorders, characterized by the proliferation of one or more blood lines with normal or nearly normal maturing in the bone marrow and in extramedullar hematopoietic organs. Hemorrhagic syndrome is a complication that occurs in about a quarter of patients with PV and even 60% patients with ET. Bleeding may complicate the clinical course of the IMF. It is manifested in the form of petechiae and ecchymoses, or may be life-threatening as uncontrolled esophageal bleeding. Bleeding occurs due to ineffective megakaryocytopenia, retention of platelets in the large spleen, qualitative
platelet disorders, acquired deficiency of factors V and VWF, disseminated intravascular coagulation, panmyelosis. 

Aims: The aim of this study is to monitor the count of erythrocytes, leucocytes and platelets, as well as hemoglobin and hematocrit values as potential risk factors for the incidence of hemorraghic 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 neoplasias. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (61); 2. Group with essential thombocytocythemia (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, leucocytes and platelets, as well as hemoglobin and hematocrit values. We used methods of clinical, laboratory, endoscopy, ultrasound and CT scans.

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 x10¹²/μL, leucocyte count 1.2-27.1 x10⁹/L and the platelet count ranged from 10.2-1986.6 x10⁹/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 leucocyte 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 of MPNs with ET 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 thombocytosis, 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 10x10⁹/L (p<0,05) and in patients with platelet count over 1000x10⁹/L (p<0,01). The increase in platelet count influences the adsorption of larger von Willebrand multimers on the platelet mem-brane, 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 leucocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasias, 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.

PB2062

CLINICAL RELEVANCE OF JAK2V617F MUTATIONAL LOAD IN PATIENTS WITH PHILIPADIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS FROM REPUBLIC OF MACEDONIA (SINGLE-CENTER EXPERIENCE)

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Background: Polycythemia vera (PV), essential thombocytocythemia (ET), and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative neoplasias, characterized by the expression of an 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. Quantification of the JAK2V617F/G617F 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 experts 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 leucocyte count (11.9±4.6 x10⁹/L vs 8.87 ±13.8 x10¹²/L and 8.9±13.9 x10⁹/L in ET, PV and PMF pts respectively), with elevated thombocytocyth 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 10¹²/L (15.45 vs 14.35 and 1071.5 vs 860.5 in ET patients respectively) (p<0,05 for all comparisons).

Background: Chronic neutrophilic leukemia (CNL) is a rare BCR-ABL1-neg-ative myeloproliferative neoplasm (MPN) with 200 patients reported to date according to the WHO criteria. These cases are characterized by a high number of mature neutrophils in peripheral blood (PB), a hypercellular bone marrow due to neutrophilic granulocytosis proliferation and hepatosplenomegaly. None standard of care exist for CNL; most patients are palliated with hydrox-urea, interferons, splenic radiation or splenectomy. In the past years CNL has been considered as chronic myeloid leukemia (CML), atypical CML (aCML) or chronic myelomonocytic leukemia (CMML), however, this diagnosis has been more defined since the oncogenic mutations in the granulocyte colonystimulating factor receptor (CSF3R) gene were identified in approximately 83% of WHO-defined CNL patients. CSF3R T618I mutation is now considered as a highly specific molecular marker for CNL that is sensitive to in vitro and in vivo inhibition by currently approved protein kinase inhibitors.

Aims: here we report a case of a 76-years old man with diagnosis of chronic neutrophilic leukemia, according to WHO criteria, successfully treated with ruxolitinib.

Methods: On May 2015 a 76 aged male patient presented at our Institution with fatigue, night sweats, neutrophilic leukocytosis (neutrophils 42.080/mmc, immature granulocytes <5%), and symptomatic splenomegaly (277x127x200 mm). Marrow biopsy was hypercellular (100%) with myeloid hyperplasia, mild myeloid dysplasia and profound erythropoietic hypoplasia; reticulin fibrosis was minimally present. Molecular profiling demonstrated no mutations of JAK2 or CALR and polymerase chain reaction (PCR) studies for (t9;22) and BCR-ABL fusion, was negative. The patient was initially treated with hydroxyurea with a provisional diagnosis of prefibrotic phase of primary myelofibrosis (PMF), but symptoms worsened and the therapy was interrupted after 9 months for progressive anemia (Hb 9.9 gr/dl) and thrombocytocythemia (82.000/mmc); meanwhile polymerase chain reaction (PCR) studies revealed the presence of CSF3R T618I mutation, suggesting diagnosis of CNL. By taking into account the activity of ruxolitinib in overt PMF, we decided to start this drug. The initial dose was 5 mg twice daily with a gradual increase in the dose to 20 mg twice daily when platelet count became normal.

Results: on a follow-up of 6 months after initiation of ruxolitinib therapy, symp-toms resolved, hemoglobin and platelet levels improved (PLT 186.000/mmc), leukocytosis persisted (WBC 24.600/mmc), and the patient achieved a dramatic reduction in spleen size (209x119x74 mm).

Summary/Conclusions: Current data suggest that constitutively active JAK-STAT signaling plays a central role in the pathogenesis of BCR-ABL1-negative myeloproliferative neoplasms (MPNs); our experience suggests that ruxolitinib in CNL patients can induce partial responses by improving marrow function (normalization of hemoglobin and platelet counts), splenomegaly and symptoms.
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 2 FL (n=12), Grade 3 FL (n=11), and DLBCL not otherwise specified (n=28). In situ FL and Grade 1, 2 FL were grouped as low-grade FL. Most patients with FL (11/13 low-grade FL and 8/11 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 haemoglobin methodical. 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/μl 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/μl). Comparison of low-grade FL, Grade 3 FL and DLBCL did not show any statistically significant difference regarding monocytes, CD4, CD8 and total T cells. Low-grade FL and DLBCL showed the highest number of differences, involving lymphocytes (257±2439 versus 149±671, p=0.001), NK cells (381±312 versus 204±167, p=0.03), the CD4/CD8 ratio (1.5±4.2 versus 2.06±1.44, p=0.002), and circulating monoclonal B cells, for both percentage (15.2±23.23 versus 1.94±5.23, p=0.001) and absolute number (869±1758 versus 18.75±64.47, 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 (66±41 versus 105±102, p=0.048). The peripheral 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±28.48, p=0.008) and number (869±1758 versus 43.36±69.91, p=0.002) of monoclonal B cells. The number of lymphocyte subpopulations was lower as well as with low-cell counts 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 MANTELE 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 PDPK1 as a therapeutic target molecule in MCL cell lines.

Methods: Four MCL-derived cell lines (MINO, Jeko-1, JVM-2 and Z138 cells), three diffuse large B-cell lymphoma (DLBCL)-derived cell lines (KPUM-MS3, 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 Ser2414. Cell proliferation was assessed by a modified MTT assay. Antibodies utilized for Western blotting was performed for evaluating protein expression levels of PDPK1, p-PDPK1Ser241, p-RSK2Ser2522, and RSK2. BX-912, a specific inhibitor for PDPK1, was purchased from Selleckchem (USA). RNA interference of PDPK1 was performed by transfection with pCMV-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 PDPK1 was activated through phosphorylation in tumor cells of all 7 MCL patient-derived specimens examined, and this was also the case in all 5 MCLCs examined and in all 5 follicular lymphomas examined. These indicated that PDPK1 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 four 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). In addition, the flow cytometric analysis revealed that the growth inhibition of MCL cells by PDPK1 blockade with BX-912 was at least partly mediated through the induction of apoptosis. As the molecular sequelle, PDPK1 blockade by BX-912 resulted in dephosphorylation of RSK2 or AKT activity or CCND1 expression was unaltered by BX-912 treatment in MCL cells. By gene knockdown of PDPK1 by RNA interference using three different short hairpin RNAs, we further validated that the reduction of PDPK1 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-192 showed additive/synergistic growth inhibitory effects in MCL cell lines.

Summary/Conclusions: Collectively, our study suggested that PDPK1/RSK2 signaling axis is the potential therapeutic target in MCL.

PB2066

THE ACQUISITION OF RESISTANCE TO BENDAUSTINE HYDROCHLORIDE INDUCES MULTIDRUG RESISTANCE IN A NOVEL MANTELLE CELL LYMPHOMA-DERIVED CELL LINE KUMP-YU1

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Background: Bendamustine hydrochloride (BH) has been one of the most potent cytotoxic moieties for mantle cell lymphoma (MCL), however, its mechanisms of action and the mechanisms for the acquisition of resistance to BH have not been fully clarified.

Aims: We tried to identify the underlying mechanisms for BH resistance to develop the strategy to overcome BH resistance.

Methods: This study was conducted in accordance with the Declaration of Helsinki and with the approval of the Institutional Review Board. Patient’s sample was obtained along with the written informed consent. We firstly established a novel MCL-derived cell line, KUMP-YU1, from circulating lymphoma cells of a 77-year-old male patient with MCL. A BH-resistant subline of KUMP-YU1 (KUMP-YU1R) was established by continuous exposure to BH with gradual escalation of its concentration from 5 μM up to 50 μM for about 8 months. Cyto genetic analysis was performed by double color-fluorescence in situ hybridization and spectral karyotyping (SKY). The comparative gene expression profile (GEP) and the ingenuity canonical signal pathway analyses between of KUMP YU1 and KUMP YU1R were performed to identify the differential gene expression pattern along with the acquisition of BH resistance. Cell viability was evaluated by a modified MTT assay.

Results: SKY analysis revealed that both primary tumor cells and KUMP-YU1 had complex karyotype including three-way translocation (8;14;11) (q24;q32;q23) (involving cytogenetic banding of CY3) or (q24;q32;q23). The parental cell line (KUMP) IC50 to BH was 20 μM in KUMP-YU1 cells, while the cell proliferation was not inhibited by up to 60 μM of BH in KUMP-YU1R cells. When compared with the parental KUMP-YU1 cells, KUMP-YU1R cells showed the partial cross-resistance against doxorubicin, mafosfamide, melphalan, and vincristine. By comparing the parental KUMP-YU1 and KUMP-YU1R, a total of 477 genes were differentially expressed in KUMP-YU1R compared with KUMP-YU1, including 312 upregulated more than 1.5-folds and 160 downregulated less than 0.67-folds in KUMP-YU1R cells. The ingenuity canonical signal pathway analysis based on the GEP results sug-
COMPARISON OF OVERALL SURVIVAL ACCORDING TO BONE MARROW ASPIRATION RESULTS IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA

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, vacuolation, and granulation including lymphoid aggregates. BM involvement in biopsy was defined as positive if at least one BM needle showed the presence 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 (P<0.001), vacuolation and granulation included lymphoid aggregates (P=0.001), and presentation 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.

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.

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 tumoral development and response to therapy, as well as to follow the therapeutic efficacy of the tumors with an invasive minimally invasive minimally invasive imaging method.

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 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) study of the therapy resistant and sensitive areas of each tumor. 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 tumoral development and response to therapy, as well as to follow the therapeutic efficacy of the tumors with an invasive minimally invasive imaging method.

Aims: The newly developed KPUM-YY1 cells and KPUM-YY1R cells deserve to be considered as good models for the study of multiple mechanisms underlying BH activity/resistance and the acquisition of BH resistance potentially leads multidrug resistance in MCL cell lines. Therefore, we can identify, localize and quantitatively predict 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 the role of BM involvement in both BM aspiration and biopsy (log-rank P=0.291; Figure 1).

Summary/Conclusions: This study revealed that the multiple molecular mechanisms overlying the development of BH resistance, therefore, the acquisition of BH resistance potentially leads multidrug resistance in MCL cell lines. Therefore, we can identify, localize and quantitatively predict 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 the role of BM involvement in both BM aspiration and biopsy (log-rank P=0.291; Figure 1).
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 lipidsomics, metabolomics and proteomics point of view. Primary analysis indicate very distinctive metabolomics and lipidomic profiles between relapsed and non treated tumors. Summary/Conclusions: Combining IVIS 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 lipidsomics, 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 expression 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-negative (IDO−). There were no significant differences in IDO expression comparing with Hodgkin's lymphoma. However, the IDO expression tended to have a better response to the 1st line chemotherapy compared to patients with positive IDO expression. The overall response rate was achieved in 71.4% (5/7) of IDO+ cases and in 92.9% (28/28) of IDO− cases. The relapse rate was higher in IDO+ cases, as was the disease-free survival (DFS). We found that nine out of 16 (56.2%) patients with 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 abnor- malities 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: Burkitt leukemia (BL) constitutes around 13.5% of pediatric malignancies, and having a high proportion of cases due to translocations involving the MYC gene to one of the immunoglobulin genes. The clinical significance of secondary chromosomal abnormalities associated with this characteristic translocation remains unknown.

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 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: Forty-seven seven BL patients were diagnosed and treated during the study period according to the FAB/LMB 96 protocol. Majorly 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. Information for 46 patients demonstrated translocation of the MYC and IGH genes in 54 patients (86%) while translocation of the IKG 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 abnormali- ties in 14 patients, and 2 abnormalities in 16 patients. All patients 1q common secondary 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 transloca- tion (6 patients), chromosome 6q deletion (4 patients), chromosome 13q deletion (3 patients), marker chromosome (3 patients), loss of chromosome 17p (2 patients), isochromosome 9q (2 patients), translocation of chromosome 13, tri- somy 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).

PB2071
IGHV SOMATIC MUTATION PROFILE AS PATHOGENETIC SIGNATURE IN SPLENIC MARGINAL ZONE LYMPHOMA AND SPLENIC DIFFUSE RED PULP LYMPHOMA.
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Background: Splenic lymphomas (SLs) are rare chronic lymphoproliferative neoplasms with a very different clinical course and a non-characteristic phenotype and karyo- type. Genetically, many cases of SMZL and SLP are related to B-cell lymphoma and characterized by a peculiar morphology with micronodular pat- tern of infiltration, biphasic cytology, and the most common presence of mar- ginal zone differentiation. Splenic diffuse red pulp lymphoma been introduced as a provisional entity but differential diagnosis with other SLs is needed to be clarified since the therapeutic approaches are distrinct.

Aims: The aim of our study to determine the immunoglobulin variable heavy chain (IGHV) gene usage and somatic mutation patterns in a series of SMZL and SLPR patients. Methods: We studied 24 patients with SMZL, 40 patients with HCL and 10 patients with SDRPL. Diagnosis was based on standard WHO classification. In 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 IGHV gene. 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 SMZL revealed that 6 cases used VH1 and in 5 cases VH2. In the 2 VH family, 16 the VH1 family segments. The VH1 family genes V1-2 were used in 16 cases. In 4 out of 24 cases (16.67%), IGHV genes were in germline or near germline 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 rate between the mutated and unmutated cases of SMZL. A comparison of the VH genes to reported germline sequences in SDRPL revealed that five cases used the VH3 family VH gene segments and five the VH4 family, one of case with unmutated IGHV genes. Summary/Conclusions: Our analysis also showed the selective use of VH1 and VH2 by SMZL cases. A high proportion of cases with VH4 family genes were represented at a lower frequency (8.33% and 25%, respec- tively). The present study may revealed that SMZL and SDRPL derive from different cellular origin and may used in differential diagnosis.

haematologica | 2017; 102(s2) | 823
Madrid, Spain, June 22 – 25, 2017
CELL OF ORIGIN ASSIGNMENT USING IMMUNOHISTOCHEMISTRY IS INFLUENCED BY BCL-2 EXPRESSION IN DLBCL PATIENTS TREATED WITH CHEMO-IMMUNOTHERAPY

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Summary/Conclusions:

Background: Diffuse Large B-cell Lymphoma (DLBCL) is a heterogeneous disease with variable clinical and pathologic presentations. Using gene expression profiling or Lymph2Cx assay, DLBCL can be assigned as germinal center (GC) 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 rituximb. 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, 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 48.6%, respectively (p=0.008) but OS was similar at 77.6% and 71.2% respectively (p=0.181). Interestingly, BCL-2 expression predicted OS irrespective of COO assignment. Patients with BCL-2 expression had a 2-year OS of 59.6% vs 56.2% for GCB and non-GCB, respectively. Whereas, patients without BCL-2 expression has a 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.

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 life-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 germinal center (GC) and post-GC B-cell origin in pediatric EBV carriers, to investigate whether an alteration of microenvironment could be related to lymphomagenesis

Methods: Formalin fixed paraffin embedded (FFPE) pediatric biopsy samples from 26 DLBCL, 55 HL and 41 tonsils from EBV carriers were analyzed. Immunohistochemistry 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 12.7±7.2 in the EBV+ carriers, 5.7±3.8 in the EBV- carriers and 5.5±3.5 in the EBV+ adenoids. CD8+ cell count were statistically higher at the GC region in EBV+ carriers compared to EBV+ lymphomas from EBV carriers. 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 no in HL. Concerning EBV, pediatric carriers did not showed differences in cytotoxic activity according to EBV presence at the CG (p=0.05, Mann Whitney test). In fact, GrB cytotoxic activity was prevalent only at the EBV+ subepithelial region (p=0.0420, Mann Whitney test).

Summary/Conclusions: Latency II pattern prevails in both pediatric EBV-associated lymphomas and in EBV+ CG from EBV 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+ cells are present at the critical 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.

PB2074

MICRAARRAY EXPRESSION PROFILE OF LONG NONCODING RNAs IN GERMINAL CENTER-LIKE DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Long noncoding RNAs (IncRNAs) are constantly transcribed and involved in a variety of biological activities. The contributions of IncRNAs to the development of germinal center (GC)-like diffuse large B-cell lymphoma (DLBCL) remain largely unknown.

Aims: The aim of this study was to investigate the expression profile of IncRNAs in human GCB DLBCL cell lines (OCI-ly1 and OCI-ly19) and normal B lymphocytes by microarray.

Methods: We used ArrayStar Human LncRNA Microarray V3.0 for profiling of IncRNAs in our specimens. Sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) with minor modifications. Quantitative
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 lncRNAs were expressed in all samples analyzed, of which 1.648 incRNAs were upregulated and 2,671 lncRNAs were downregulated in GCB DLBCL cell lines (OCI-Iy1 and OCI-Iy19) (≥2.0-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 the potential target genes related to the 3 upregulated and 2 downregulated lncRNAs.

**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
FLOW 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 hematolymphoid neoplasms occurring at extranodal sites. Flow cytometry at extranodal sites cannot present 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 specimens or fine needle aspiration cytology (FNAC). For years FNAC has been used for initial evaluation of suspected hematolymphoid neoplasms. Flow cytometry can additionally help in identifying B or T cell nature of neoplastic cells, clonality in case of B-cell neoplasms and a aberrant phenotype. The possibility of detecting CD20 status can help in initiating targeted therapy without undergoing tissue biopsy to do so. FNA cytology with Flow cytometry can serve as a replacement for open biopsy and may help in eliminating the need for more invasive procedures. In this study FCM analysis on cytological specimens, including nodal and extranodal mass from GIT, Thyroid, Kidney, Breast, Tonsil, 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.

**Methods:** The current study was prospectively conducted 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 (IHC) evaluation. Samples collected in Isotone were sub-mitigated for FCI on 5-color Beckman Coulter FC-500, using a set of mature and immature antigens markers for lymphoid cells. Results of FCI on cytological specimens along with cytomorphological findings were compared with histological and IHC diagnosis.

**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`s/PNET (1 case). Combining FCI with cytological findings definite diagnosis could be found in 33/40 (82%) cases compared to 30/40 (75%) cases, hematolymphoid malignancies of solid biopsies were sub-mitigated. As per World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues 2008 most common lymphoma at extranodal site was DLBCL followed by acute lymphoblastic leukemia/lymphoma. Whereas at nodal site most common lymphoma was DLBCL followed by Hodgkin`s lymphoma. The estimated diagnostic sensitivity of FCI alone was 60.5% with 50% specificity, similar for both extranodal and nodal tissue samples. Whereas after combing FCI with cytological findings sensitivity and specificity was found to be 78.4% and 100% respectively. Immunophenotyp- ing of lymphoblastic leukemia/lymphoma by FCM on cytological specimens was found to be in 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 at 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 markers (CD38) and the subset CD4+CD26-CD38+ to identify the neoplastic cell population in HL. A subset connectable to regulatory T (Treg) cells, because the low expression of CD26 (DP4) added to the presence of CD39 (NTPDase) may be responsible for the generation of adenosine, which plays a major role in T-regulated immunosuppression.

**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 analyze 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+CD39+ 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 CD T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target.

PB2077
TREG CD4 PHENOTYPE IN THE PERIPHERAL BLOOD OF LYMPHOMAS
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**Background:** The T regulatory (Treg) cells down-regulate antitumor responses by several distinct mechanisms. One is the adenosinergic pathway which, through ectonucleotidases, sequentially converts ATP to AMP and generates adenosine. Adenosine is one of the most important immune suppressive adenosine deaminase inhibitors (AIDA) levels, the enzyme responsible for adenosine breakdown, and of CD26, a surface-bound ADA associated glycoprotein. In our previous work (Di Gaetano et al, Ann Haematol, 2014) we analyzed by flow cytometry (FC) the CD T lymphocytes of solid biopsies, the surrounding stromal cells in Hodgkin lymphoma (HL) lymph nodes (LN) and we demonstrated the presence of an activated profile (CD38+) with a reduction of CD26 (CD4+CD26-CD38+). We also confirmed a link of this subset with an overexpression of CD39.

**Aims:** By using the same FC technique we wanted to explore if, as in the lymphoma tissue, the CD4 T regulatory profile in the peripheral blood (PB) of HL at diagnosis and possibly to distinguish them from those of B Non-Hodgkin lymphomas (B-NHL).

**Methods:** We have analysed by FC the PB of 16 healthy controls (HC), 10 HL and 22 NHL testing within T CD4 cells the expression of CD26, CD38 and CD39.

**Results:** In HC CD26+CD38+ cells were 2.6% of all CD4 and 5.5% expressed CD39. Compared with HC, the subset CD4+CD26-CD38+ of HL was statistically different (2.6 % vs 17 %, p<0.05) as well in B-NHL (2.6 vs 12.9; p<0.05). The expression of CD39 between HC and HL was not different (5 vs 9.8; p=0.1), while it was statistically significant between HC and NHL (5 vs 19.5; p<0.05).

**Summary/Conclusions:** Our results may suggest that T CD4 profile in the PB can characterize the patients with HL and B-NHL and this could be probably variable according to the type of neoplasia. The significant presence of CD26 in HL and B-NHL would seem to suggest that the low expression/reduction of CD26 of ADA activity may indicate the T-regulated immunosuppression. Interesting is the diversity of NHL showing increased CD39 expression on T CD4 lymphocytes probably connected with
the clone of B lymphocytes involved in cancer. This may support that leukemic cells may contribute to create adaptive immune responses in a cancerous environment and to facilitate immune escape mechanisms. FC analysis of CD26 and CD39, markers likely involved in the adenosinergic pathway, in PB26 can represent effective parameters to determine and characterize the Treg CD4 different in 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-cell 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 completely accurate prognostic information so the International Prognostic Index (IPI) which identifies poor- and good-risk patients with diffuse large B cell lymphoma (DLBCL) is still part of all current diagnostic guidelines; however, the majority of patients have an intermediate IPI, with an uncertain prognosis.

Aims: In this study, we investigated the impact of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI as well as impact of GCB and non-GCB subclassification according to Hans and Muris’s algorithm 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 or favorable group 1 and unfavorable group 2. According to Hans’s algorithm and Muris’s algorithm. Clinical-pathological, immunohistochemical algorithms for identification of these phenotypes have been used with subphenotype BCL-2, B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and others. PML-CHL, B-cell lymphoma, and mediastinal CHL, mostly nodular sclerosis (NS) share many clinicopathologic characteristics, however, therapeutic options and responses are quite different.

Aims: We aimed to find distinctive histologic or immunohistochemical findings to better differentiate PMLBCL and CHL of the mediastinum.

Methods: A total of 32 cases of mediastinal B-cell lymphomas consisting of PMLBCL (N=16), CHL (N=13), and gray zone lymphoma (N=3) were collected from 6 university hospitals from Korea. Immunohistochemistry (IHC) for various cell lineage markers and EBV in situ hybridization were performed to confirm the diagnosis, and additionally, expression of P63, GATA3 and cyclinE was investigated.

Results: Most clinical features were overlapped between PMLBCL and CHL except more frequent disease progression and mortality in PMLBCL (p<0.05). In pathologic review, presence of epithelioid granuloma favored CHL (p=0.078), whereas fine reticulated fibrosis was unique for PMLBCL (p<0.001). By IHC, P63 was predominantly positive in PMLBCL (15/16) than CHL (2/11) with the highest diagnostic power (p<0.001). GATA3 was expressed in the majority of CHL (9/12) compared with PMLBCL (0/16) (p<0.001). Expression of cyclinE was rarely found in a minor population of PMLBCL.

Summary/Conclusions: Expression of P63 in the tumor cells, even focal, is the most helpful feature to distinguish PMLBCL from mediastinal CHL. Additional diagnostic markers include GATA3 in CHL and reticular fibrosis in PMLBCL.

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 microves- sel density and the subtype of the disease has not been established yet. We aim 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 immunomicroarchitecture with typical angio-fibulic 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 visualized by CD34 antibodies. Slides were scanned with Pannoramic 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±1,0% of the area. In patients with plasma cell variant percentage of blood vessel area was higher 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 plas- ma cell variant.

Summary/Conclusions: The highest index of vessel density in the lymph nodes of each variant was observed in hyaline vascular CD. 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 for CD10, as well as positive expression for 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.

**Other Non-malignant hematopoietic disorders**

**PB2082**

**LYMPHOID NEOPLASMS: A REALLY IMPORTANT TRIGGER IN HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS**

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**Background:** Triggered by several conditions Hemophagocytic lymphohistiocytosis (HLH) is an unusual, aggressive and life-threatening dysfunction caused by an excessive immune activation. It has become more recognized over the past decade. HLH was first described in 1939 by Scott and Robb-Smith, next case was reported in 1952 by Farquhar and Claireaux describing two infant siblings with progressive and lethal cytopenias, hepatosplenomegaly, and fevers with autopsy showing hemophagocytosis. A lengthy and unstoppable activation of antigen-presenting cells (macrophages, histiocytes) and CD8+ T and NK cells is characteristic. This condition leads to an important hyperinflammatory situation and organ damage including splenomegaly, fever, cytopenia, hypertrigliceridemia and/or coagulopathy. Histiocyte Society (HS) criteria have been applied for diagnosing HLH, however not all of them are usually showed at the presentation. This disease can be described in two different scenarios: primary (usually in children, genetic, and known as familial form) and secondary (acquired). It can be triggered by a large variety of events that disrupt immune homeostasis. When we talk about triggers, we can divide them in two broad categories, those that cause immune activation and those that lead to immune deficiency. Lymphoid neoplasms can be both.

**Aims:** Due to the lack of publications about HLH secondary to Lymphoid Neoplasms (LN), we would like to analyze the casuistry of our hospital and making a comparison with the current literature.

**Methods:** We conducted a retrospective analysis through medical files of all patients with suspected diagnosis of HLH between 1994 and 2017 in our inpatient ward. Clinical features, age, diagnostic criteria proposed by the HS, etiology, treatment and evolution were analyzed. In our study 18 out of 50 patients met the requested criteria for HLH diagnosis.

**Results:** We report 10 LN secondary cases (4 males, 6 females). The median age at diagnosis was 60.5 years, ranged between 46 and 80 years. In all of them, but in one, who presented long-term pancytopenia, symptoms were developed very fast. The most frequent causes of consultation were cytopenia and general syndrome. In two of them HLH was diagnosed with LN relapse, in one patient during a transformation from a low-grade B-cell lymphoma to DLBCL (Diffuse large B-cell lymphoma), in 6 of them we diagnosed LN and HLH concomitantly, and in the last one coinciding with a Richter Syndrome. Four of 10 were secondary to T-cell neoplasm. All patients met 5 or more HS diagnostic criteria. In only 3 of them HLH was healed. One patient is still in remission. Nine died, 7 of them due to HLH complications. Treatment was chemotherapy (depending on their LN) in almost all of them. Fluctuations were detected among activity HLH parameters due to LN response. Detailed characteristics of patients are shown in Table 1.

**Table 1.**

**haematologica | 2017; 102(s2) | 827**

Madrid, Spain, June 22 – 25, 2017
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 he importance of early diagnosis. Despite being a serious, rare 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 preclude evaluation 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 level when symptoms or clinical features of lymphoid neoplasm are not concordant with the expected evolution.

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. The main goals of these networks is to facilitate the exchange and 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 to disseminate more information to the general public on these 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 (PNDs) as well as international guidelines. The network will also be setting up new relationships with the general public and hematological societies involved in 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 multidisciplinary 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 organised on January 30th 2016 in Paris to inform on the update status of research on their disorders 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 of new registries 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 piloted 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 futher 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 thus ascertained that TMA was due to carcinoma unknown primary. The second patient presented with general neurological findings like Guillain-Barre Syndrome and paraplegia 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 anemia, thrombocytopenia, fever and pneumonia findings. We conducted PLEX therapy. On the 8th day of PLEX, the patient had anaphylaxis, we performed cardio pulmonary resuscitation. The fourth patient
presented with acute renal failure with malignant hypertension. We performed hemodialysis together with PLEX treatment. Because his diagnosis was acute renal failure, malignant hypertension, and TMA. The fifth patient presented with epistaxis and sepsis. He had chronic TTP diagnosis from two years ago. We diagnosed 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 presented with a fever that had been going on for five days. We treated the patient with PLEX together with the corticosteroid. Because his ADAMTS 13 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 pulsed 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 malignant hypertension-induced renal failure was successfully treated with PLEX, hemodialysis and antihypertensive treatment. We successfully treated our bacterial infection and sepsis-induced TMA 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 shichyocyte count and atypical neurological findings. ADAMTS 13 activity may only be a guide for diagnosis of TTP, but it is unreliable for a definitive one. In conclusion, diagnosis of TTP and other TMAs is difficult. Etiology, clinical features, laboratory findings should all be taken into account when diagnosing TMA. While it is established that ADAM TS 13 deficiency is the major cause in acquired TTP, finding the etiology of other TMAs is determinant for a successful treatment of the latter.

PB2085

HAEMOLYSIS AS SCREENING TEST IN LYSSOSOMAL 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 incidence of hematological findings in LSDs 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 showed that haemolysis at 0.48% of NaCl allow us to sort out controls vs LSDs-C/LSDs-P (AUC=0.725) whereas no significant differences 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 abnormality. Elevated serum cobalamin levels may be a sign of a wide range of diseases like solid tumours, haematological disorders like leukemia, storage disorders, chronic myelogenous leukemia, promyelocytic leukemia, polycythemia vera, hyperesinophilic syndrome as well as liver and kidney diseases.

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 Hacettepe 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, neutropenia 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 patients (Acute lymphoblastic leukemia (ALL) n:1, acute myeloblastic leukemia (AML) n:1, large granular lymphocytic leukemia (LGLL) n:1), myelodysplastic syndrome (MDS) in 2 patients, isolated thrombocytopenia in 4 patients, isolated neutropenia in 7 patients, bicipytopenia in 4, aplastic anemia in 2 patients, cobalamin metabolism defects in 10 patients, hemoglobinopathies in 2 patients, polisitemia in 1 patient, cystic fibrosis in 1 patient, HIV in 1 patient, FMS (familial mediterrenian fever) in 1 patient, chronic kidney failure in 2 patients, sickle cell anemia in 1 patient, factor V Leiden mutation in 1 patient, hyperesinophilia 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 the 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 findings 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, hematological 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, homocystechagocytosis, 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 determined, 127 anemia of chronic disease, 80 iron deficiency anemia, 56 cytopenia and four vitamin B12 deficiency anemia. Leukocytosis (n=1), thrombocytosis (n=5) and homocystechagocytosis (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, pancytopenia may be diagnostic findings in the early stages of the disease. Metabolic diseases 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-
Results: was HemosIL SynthASil-SS which was sensitive against only plasma factors. It's sensitive against both plasma factors and lupus anticoagulant. It contains mix using three different reagents. First reagent was HemosILAPTT-SP which was included as yet. Patient's APTT levels were calculated ACL-TOP analyzer by We tried to show the importance of APTT reagents and how to reach and by eliminating the lupus antıcouglant from plasma. This was caused to procedure by detecting the mild or moderate deficiencies of plasma factor levels We should detect these kind of diseases and we should examine the correct diseases which had bleeding events in surgical procedure or spontaneously. We need further studies to make a standardization and harmonization of APTT.

Summary/Conclusions: Haemostasis is a complex physiological cascade which was began at the endothelium injury. Many kinds of complex procedures occurred with this injury. 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. Therfor sensitive of APTT reagents are very important. Every kind of APTT reagent do not have equal sensitivity against plasma factor factors, phos- pholipids, protein C, protein S and protein Z in thrombin time. Several stud- ies suggested that range of APTT should be determined according to the devices and reagents and also several studies compared APTT reagents which was included silica, elagic acid and phospholipids by composed of synthetic or animal orginaged 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 borderlines 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.

Results: bin (MCH) were evaluated. (RBC), leukocytes (WBC) and platelets (PLT), mean corpuscular hemoglo- ples for hematological measurements were examined at 4, 9, 13 weeks after treatment. Different doses of 0; 10; 50; 200 mg/kg/bw/day were defined and were the same in all studies. Blood sam- ples for hematological measurements were examined at 4, 9, 13 weeks after TB exposure in the same groups of animals throughout the experiment. Hemo- globin (HGB) concentration, hematocrit (HCT), total amount of erythrocytes (RBC), leukocytes (WBC) and platelets (PLT), mean corpuscular hemoglo- bin (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 5th 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. Some of generic TBs lead to decrease (leukopenia) or increase (leukocytosis) of leuko- cytes 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 impor- tant to investigate the hazardous effects of pesticides on the blood system.

What we can do to make a standardization and harmonization of APTT?

Aims: To try 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 yet. APTT levels were calculated ACL-TOX analyzer by using three different reagents. First reagent was HemosILAPTT-SP which was sensitive against both plasma factors and lupus anticoagulant. It contains mix collodial silica and normal range of APTT- SP was 25.4-36.9 s. Second reagent was HemosIL SynthAASi-SS which was sensitive against all plasma factors. It contains mix silica and normal range of APTT-SP was 25.1-36.8 s. The third reagent was HemosIL Syntha-FOX-SS which was sensitive against only lupus anticoagulant. It contains ellagic acid and normal range of APTT-SS was 21.5-30.4 s.

Results: Forty-five of 109 patients had normal level of APTT by measuring three types of reagents. Seventeen of 109 patients had long level of APTT by measuring Hemosil SynthAASi-SS reagent and had normal level with Hemosil SynthaFox-SS and Hemosil APTT-SP. Seventeen of 109 patients had long level of APTT by measuring Hemosil SynthAASi-SS reagent and had normal level with Hemosil SynthaFox-SS and Hemosil APTT-SP. Seventeen of 109 patients had long level of APTT by measuring Hemosil SynthAASi-SS reagent and had normal level of APTT with Hemosil SynthaFox-SS and Hemosil APTT-SP.

Summary/Conclusions: Haemostasis is a complex physiological cascade which was began at the endothelium injury. Many kinds of complex procedures occurred with this injury. 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. Therfor sensitive of APTT reagents are very important. Every kind of APTT reagent do not have equal sensitivity against plasma factor factors, phos- pholipids, protein C, protein S and protein Z in thrombin time. Several stud- ies suggested that range of APTT should be determined according to the devices and reagents and also several studies compared APTT reagents which was included silica, elagic acid and phospholipids by composed of synthetic or animal orginaged 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 borderlines 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.

Acquired pure red cell aplasia in an adolescent: could it be anything else?

Aims: We report a 16-year-old male with acquired pure red cell aplasia who was treated successfully with steroids and cyclosporine after elimination of the secondary causes.

Methods: Case: An 16-year-old boy presented with a history of pallor and fatigue noticed three months prior to admission. He had been diagnosed with immune thrombocytopenia when he was 5 years old and had been in remission since that time. There was no history of blood transfusion, chronic illness or any other medication. His physical examination revealed pallor and a 2/6 sys- tolic murmur with no other abnormalities. Complete blood count revealed severe macrocytic anemia and reticulocytopenia with hemoglobin: 2.2 g/dl, hematocrit: 0.2%, mean corpuscular volume:108.7 fL, red blood cell: 0.57x10^{12}/L, reticulocyte: 0.2% and mild leukopenia and lymphopenia.Periph- eral blood smear showed macrocytic red cells with occasional tear drop cells. Stool for occult blood was negative. The direct and indirect antitubulin tests were negative Serum bilirubin, LDH, haptoglobin, liver function tests and renal function tests were in normal limits. Hemoglobin F was 2.9%. Bone mar- row aspiration showed red cell hypoplasia, without dysplasia or giant pronor- moblasts and normal myeloid and megakaryocytic series. A high resolution computed tomography of chest ruled out thymoma. Serumin immunoglobulins revealed low IgA with normal IgG and IgM levels. Anti-nuclear antibody was positive. Anti-DNA test was negative. Parvovirus B19 DNA and other serologic markers including antibodies to HIV and hepatitis A, B and C were all negative. He was transfused with erythrocytes and discharged with a hemoglobin value of 7.2 g/dl. On his follow-up, hemoglobin levels were observed to decrease again. A diagnosis of primary acquired PRCA was considered and prednisone was started with 1 mg/kg/d and increased to 2 mg/kg/d. After 2 months of therapy, hemoglobin level rapidly increased and reached to 12 g/dl and leukopenia and reticulocy- topenia resolved completely. Prednisone was tapered after 4 weeks and stopped. He is still on cyclosporine treatment and has been transfusion free with stable hemoglobin levels in the second month of his treatment.

Results: Primary acquired PRCA is very rare in childhood, secondary causes must be eliminated for definitive diagnosis. Our patient was found to have lym- phopenia, low immunoglobulin A level and positive antidNA in further inves- tigations, yet these results are not sufficient for a specific diagnosis like common variable immunodeficiency. We have treated the patient with prednisone, and we considered primary acquired PRCA as the most possible diagnosis and started immunosuppressive therapy; his clinical follow-up will probably give us further details about the underlying disease.
Summary/Conclusions: Immunosuppressive therapy including cyclosporine with or without steroid has been reported as the most effective treatment in primary acquired PRCA. Consistently, we had a dramatic response to immunosuppressive therapy in our patient.

PB2091
APLASTIC ANEMIA IN CHILDHOOD: A TEN YEARS’ SINGLE CENTER EXPERIENCE
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Background: Aplastic anemia in childhood is a rare, life-threatening disorder, characterized by peripheral blood pancytopenia and a hypocellular bone marrow without signs of dysplasia or fibrosis. Acquired aplastic anemia needs to be distinguished from inherited bone marrow failure syndromes or myelodysplastic syndromes.

Aims: The aim of this study is to assess the clinical and laboratory findings at the time of diagnosis, the treatment approach and the outcome of children with aplastic anemia treated in our department during the past decade.

Methods: This retrospective study evaluated 9 children with aplastic anemia, who were treated and followed up in the Pediatric Department of AHEPA, during the period 2006–2016.

Results: We identified 9 children with aplastic anemia. The patients’ population included 6 (66.7%) males and the mean age at admission was 9.7 years. At the time of diagnosis, the average neutrophil count was 750/mm³, the Hb count was 8.4mg/dl and platelets count was 8770/mm³. In all of our cases aplastic anemia was acquired, expect one case of Fanconi anemia. Predisposing risk factors (including drugs exposure, viral infections, chemicals) were identified in 4 patients. Among the 9 studied patients, 3 (33.3%) had very severe, 2 (22.2%) had severe and 4 (44.5%) had very severe aplastic anemia. All of the patients received immunosuppressive therapy (consisting of antithymocyte globulin, cyclosporine A and steroids), 2 remained transfusion independent, 4 underwent bone marrow transplantation—2 from a matched related donor and 2 from a matched unrelated donor. One patient with refractory disease received, as an alternative first line therapy, eltrombopag. Complete response was achieved in 22.2%, partial response was achieved in 22.2%, relapse occurred in 11.1% and 44.5% of the patients had refractory disease. The overall survival was 77.8%.

Summary/Conclusions: A remarkable progress has been made during the past decades in the understanding of pathogenesis and management of children with aplastic anemia. Bone marrow transplantation from a matched related donor is the recommended first line therapy resulting in an excellent survival rate that exceeds 90%. In the future the development of targeted strategies for patients with the above described phenotype will further improve outcome and diminish the disease’s late complications.

PB2092
CAUSES OF IRON DEFICIENCY ANEMIA IN THE HEMATOLOGY CLINIC – SINGLE CENTER EXPERIENCE
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Background: Iron deficiency anemia (IDA) is the common nutritional deficiency worldwide. The studies concerning various causes of IDA in adult men are rare, although it is assumed that chronic gastrointestinal blood accounts for the majority.

Aims: Of the study is to evaluate retrospectively adult men with IDA that were hospitalized in our Hematology Clinic.

Methods: Two hundred fifty nine male with IDA were enlisted at this study from January 2005 to december 2015. Anemia was defined as Hg <13g/dL using the WHO criteria. IDA was considered present if serum ferritin was 15 ng/mL combined with serum iron concentration <30ug/dL with a transferrin saturation of <10%. Complete physical examination, the history of the disease and fecal occult blood test (FOBT) of three spontaneously passed stools was done in all patients. All patients had complete blood count, serum and total iron binding capacity, and a serum ferritin level. Most patients underwent esophagogastroduodenoscopy (EGD). Colonoscopy was performed if lesion that caused IDA was not found, and/or FOBT was positive. The abdominal CT scan were performed according to clinician’s recommandation together with other tests related with blood lost.

Results: The median age was 62 ( range 32 to 85 ) years old. 168 of 215 (78.13%) men with IDA had symptoms such as fatigue, dizziness, or digestive complaints. The history of prior gastrectomy, hemorrhoid, that probably had caused IDA were reported in 32 (14.88%), 43 (20.0%), patients, respectively. FOBT was positive in only 65 (30.23%) subjects. 170 (79.06%) patients underwear EGD. The most common findings from EGD were gastritis (48 patients) and peptic ulcer (39 patients). Seventy eight (36.27%) patients were found to have upper gastrointestinal disorders ( 20 patients with erosive gastritis, 19 gastric ulcer, 16 duodenal ulcer, 23 gastric cancer. Eighty-nine (41.39%) patients underwent colonoscopy. That showed 44 clinically important lesions that probably caused IDA; colon cancer in 17 (7.90%) patients, colon polyp in 10 (4.65%) patients and hemorrhoid in 17 (7.90%) patients. Concerning malignant lesions which are responsible for IDA, the malignant lesions were found more frequent in patients older than 50 years accounting for 20.45% (27/132 patients) and patients younger than 50 years 17.80% (13/73 patients).

Summary/Conclusions: This study demonstrated that gastrointestinal blood loss is the main cause of IDA in adult men, and that there is a high rate of malignancy in men older than 50 years.

PB2093
IMPACTS OF CLINICAL AND BIOCHEMICAL PARAMETERS ON KEY HEMATOLOGICAL INDICES IN ADULTS: A COHORT STUDY
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Background: Studies in Caucasians have shown that values of hematological parameters could be affected by a wide variety of factors, most notably age and gender. However, parallel work in different ethnic populations, especially from Asia-Pacific region, is lacking. Importantly, it remains largely unknown whether some fundamental variables such as nutritional status, lipid profile, and hepatitis infection (either HBV or HCV) also affect the variation of values in hemogram.

Aims: Therefore, we conceptualize this study to explore through several key parameters regarding their potential impacts on the changes of hemogram.

Methods: Adult individuals aged 18 or older from several adjacent villages in Yun-Lin County, located in the central part of western Taiwan, who came to our hospital for annual health exams were screened for the current study. The work, retrospective in nature, was approved by institutional IRB. Information on age, gender, hemogram, levels of total cholesterol (TC), triglyceride (TG), apolipoprotein B (Apo B) as well as albumin, and results of serological testing for hepatitis B and C infection, was obtained from a centralized digital data base. All the clinical data, after given a coding number for each case, were encrypted and provided to the investigators without identifiable personal information. We analyzed the impacts of various parameters on several key hematological indices.

Results: Overall, 26,497 individuals were included in the current analysis after excluding those with hemogram data fell outside of normal range. Carriers of either hepatitis B (HBV) or C (HCV) who had abnormal liver function (defined by elevated levels of aspartate transaminase or alanine transaminase) were excluded as well. Age, gender, and serum levels of TC, Apo B, and albumin all significantly impacted most key hematological profiles. As the levels of TC and Apo B correlated well with each other (correlation coefficient r=0.82211, p <0.0001, Pearson’s correlation), we did not incorporate TC in our multi-variate analysis. Several key variables were found to influence some hematological indices in the multi-variable regression model. Increasing age and male gender negatively affected the platelet count, whereas higher Apo B level was associated with elevated platelet count. Surprisingly, hepatitis C carriers with normal hepatic function had slightly higher platelet number than non-HCV carriers. Gender and serum albumin level were the major determinants of variation in hematological parameters. Total white cell count increased with male gender and elevating Apo B level but was inversely correlated with change in age and serum albumin level (Table 1).

Table 1.

Summary/Conclusions: The hematological indices are influenced by a wide variety of factors, especially age, gender, and serum level of Apo B. As age,
Apo B, while cell count, and platelet count all impose risk of thromboembolism, further work exploring the interactions 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).

Aims: To establish the features of genotypes distribution for several cytokine promoter gene polymorphisms in ITP patients from NWR.

Methods: 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 (4 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)-1b (-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 -592C allele was more often seen in patients diagnosed before 50 years of age in comparison with other patients: 15.8% vs 8.2% respectively; OR=2.1, 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 (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 -592C allele is associated with increased risk of ITP in women from 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.

PB2095

COMBINED TREATMENT OF AZATIOPRINE 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 8% failure has been described. In recent literature, there are clinical cases and small series describing the potentiating effect of combined treatment with thrombopoetin 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 30x10^9/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 x10^9/L and/or to avoid corticosteroids withdrawal. We have included patients with acute or newly diagnosed ITP refractory to corticosteroids or corticosteroid-dependence and refractor to steroids and azathioprine. The initial doses of romiplostim monotherapy were: • Median corticosteroids dosage 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 NF. • 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 6x10^9/L (2-13x10^9/L, IQR). The median platelet count achieved at maximal doses of romiplostim for at least 2 consecutive weeks was 10x10^9/L (3-19x10^9/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 type of response were: • One patient did not respond after 60 days of corticosteroids and romiplostin treatment. • 1 patient with RC maintains for 7 months in the absence of active treatment. The combined was necessary during 6 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-ontology 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)-X; 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 0.29 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-X; 0.59 [0.37-0.94] for PAR-1-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: The examined-induced platelet reactivity was significantly correlated to bleeding. Platelet function testing could provide a basis for a personalized transfusion regimen, 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

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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 and chronicity of the disease. It was determined that the field 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 the homozygous TNF-α G allele 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 affect the pathogenesis of ITP and facilitate the development of new therapeutic modalities are recommended.

PB2098

PROGNOSTIC FACTORS IN PRIMARY IMMUNE THROMBOCYTOPENIA OF CHILDREN

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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. Unfortunately, few studies have yet been performed 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 to 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 10⁹/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) and had received more than one 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

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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 diagnosis, the examination of bone marrow 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.

Summary/Conclusions: The CAIT is a rare hematological paraneoplastic syndrome that occur in solid tumors, usually associated to low platelet count but without life-threatening bleeding, requiring therapy with corticosteroids as first line, and generally related with a benign clinical course with a rapid and persistent response.

PB2100

THE ROLE OF MEAN PLATELET VOLUME IN NEONATAL SEPSIS: AN INTERVAL PROSPECTIVE CASE CONTROL STUDY IN A LEVEL III NEONATAL INTENSIVE CARE UNIT

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Background: Mean platelet volume (MPV) is an index of mature platelets and reflects the differences in platelet size distribution. It is well known that MPV is a good marker of sepsis. However, MPV has not been studied in neonatal sepsis. To assess the role of MPV in neonatal sepsis, this study has been undertaken.

Methods: This was a prospective observational study conducted in a tertiary care NICU. A total of 100 preterm neonates admitted to NICU with gestational age less than 37 weeks and postnatal age of 2 days were enrolled. Clinical examination and laboratory investigations were performed. MPV was measured using an automated Coulter counter (B.K. Instruments, USA). The data were analyzed using chi square test and student’s t-test.

Results: Out of 100 neonates enrolled, 50 neonates with sepsis and 50 with no sepsis were included. The mean age of neonates was 6.25 ± 0.98 days. Mean MPV of neonates with sepsis was 9.57 ± 2.97 fL and 7.87 ± 2.24 fL in neonates without sepsis. MPV was significantly higher in neonates with sepsis compared to neonates without sepsis (p = 0.04). The area under the curve for MPV was 0.68. The sensitivity and specificity were 57.6% and 78.8% respectively. The positive and negative predictive values were 77.6% and 57.6% respectively.

Summary/Conclusions: The study showed that MPV can be a useful marker for early diagnosis of sepsis and can be used as a decision-making tool in the NICU.

haematologica | 2017; 102(s2) | 833

Madrid, Spain, June 22 – 25, 2017
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 Hypoxic Ischemic 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 according to clinical sepsis (as 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, CRP-reactive protein, immature to total neutrophil count and mean platelet volume were all drawn on 2 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 0.2 in the 24-48 hour 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).

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. Lowe1, 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 <50 x 10^9/L at presentation however, National BCSH Guidance advises against platelet transfusion in TTP due to the perceived high aggregability of platelets and risk of associated fatal thrombosis. The risk of thrombotic thrombocytopenia related haemorrhage however creates 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 to determine the average platelet count at time of line insertion and to observe 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 25x10^9/L (IQR 9-26 x10^9/L). 70% of lines were inserted by critical care and the remaining 30% by interventional radiology. Platelet transfusion was not administered pre line insertion and no line insertion 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 thrombotic thrombocytopenic 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. Kalciu1,*, E. Gavriilaki1,*, G. Papaioannou1, Z. Bousiou1, M. Iskas1, C. Vadikolou1, C. Lalayanni1, A. Athanasadou1, R. Saloumi1, 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 the 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 agents.

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 ≥100x10^9/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), cyclophosphine (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 and stopped. There was however, a 22% 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 0.2 in the 24-48 hour 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).

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.

PB2103

VITAMIN D RECEPTOR GENE POLYMORPHISMS IN ADULT PRIMARY IMMUNE THROMBOCYTOPENIA M. Sakr1

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Background: Recently, several studies have demonstrated the role of vitamin
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 assess the association of 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). Deoxyribonucleic acid (DNA) samples were extracted from peripheral blood of 40 ITP patients and 60 geographically and ethnically matched healthy controls.

**Results:** Statistically significant difference was found in the BsmI polymorphism between ITP patients and controls ($\chi^2=8.77$, P value=0.01). The BsmI polymorphism B allele was higher in ITP group than that in controls but in statistically insignificant difference ($\chi^2=2.125$, 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 blood 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 pregnancy. 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 increasing to 2g/kg at 32 weeks, 32 weeks starting with 1g/kg and 2g/kg at 32 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 start 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 or 32 weeks. For high risk pregnancies defined as confirmed antibody positive with previous intracranial haemorrhage (ICH) after 28 weeks: 36% of centres start 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 or 32 weeks starting. 60% of centres use a very high risk protocol (ICH before 28 weeks: 36% of centres start 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 or 32 weeks). Thirdly how many groups do you define after risk stratification? The A group also associated lower platelet counts and more patients pertaining to this group relapsed in comparison with the B group.

**Summary/Conclusions:** In chronic ITP, increased levels of ROS are associated with elevated autoantibody 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 platelet count nor site of bleeding.

PB2106

**IMMUNE THROMBOCYTOPENIA AND PREGNANCY: A SPANISH CASE SERIES OF 270 PREGNANCIES IN PRIMARY ITP**


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**Background:** Evaluation of reactive oxygen species (ROS) levels in chronic ITP and also a possible association between Helicobacter pylori (H. pylori) infection and immunological peripheral platelet destruction. Recent studies have shown increased reactive oxygen species (ROS) levels in 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) hospitalised in the Clinic of Haematology, 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). Methods to be used were 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 in both groups (between 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 with the B group.

**Summary/Conclusions:** In chronic ITP, increased levels of ROS are associated with elevated autoantibody 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.

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. Recent studies have shown increased reactive oxygen species (ROS) levels in 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) hospitalised in the Clinic of Haematology, 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). Methods to be used were 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 in both groups (between 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 with the B group.

**Summary/Conclusions:** In chronic ITP, increased levels of ROS are associated with elevated autoantibody 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.
50.8% of women received corticosteroids, immunoglobulins (IVIG) (16.9%), rituximab (6.8%) and/or splenectomy (8.4%). The ITP treatments between or before new pregnancies. On the other hand, 26.4% of women needed treat-
ment 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) in 73 (27.0%) women who achieved less than 30 x 10^9/l. 56 (20.7%) women exhibited hemolytic symptoms, being 30 (11.1%) of them severe bleedings.

Regarding type of delivery, this was vaginal in 63.4% of pregnancies and cesarean sections 30.5%. Median platelet count at delivery was 110 x 10^9/l (IQR, 78-181). 43 patients (23.4%) experienced 57 bleeding episodes. We only observed 48 cases (20.4%) of neonatal thrombocytopenia and therefore, newborn bleeding is low.

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 pri-
mary when an underlying etiology cannot be identified and secondary when a certain etiology exists. Data concerning ITP characteristics at a national level are needed.

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: Patient 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 for national and regional base usage considering hospitals as the core unit. A centralized platform allows access to a platform where he/she can record and study patients’ data. The entire project has been developed using the robust open source tools of operating systems and Relational Data Base Management System (RDBMS) packages.

Results: We analyzed data from 696 adult ITP patients registered from 14 different centers in Greece. The median age at diagnosis was 53 years (range 15-97 years). Two peaks were observed at the age of 19-30 and 71-80 years. There was a female (60.89%) versus male (39.11%) predominance with higher frequency of females in younger (19-30 years) and of males in older (71-80 years) ages. Females appeared with more severe thrombocytopenia. The mean 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) in 73 (27.0%) women who achieved less than 30 x 10^9/l. 56 (20.7%) women exhibited hemolytic symptoms, being 30 (11.1%) of them severe bleedings.

Regarding type of delivery, this was vaginal in 63.4% of pregnancies and cesarean sections 30.5%. Median platelet count at delivery was 110 x 10^9/l (IQR, 78-181). 43 patients (23.4%) experienced 57 bleeding episodes. We only observed 48 cases (20.4%) of neonatal thrombocytopenia and therefore, newborn bleeding is low.

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.
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PB2109

NOVEL TECHNIQUES FOR MONITORING GALNIZMANN 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 care. Due to the rarity of disease there is paucity of data regarding platelet transfusion protocols during the peripartum 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 Rotary 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 activation, were sampled and followed till 7 days post teeth extraction.

Results: Prior to teeth extraction upon injection of local anesthetics patient developed a buccal hematoma probably owing to local blood vessel penetration. The patient did not experience any post extraction bleeding. Hematoma was absorbed within several days. Post transfusion platelet counts FC demonstrated 20.6% donor platelet 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 plantlets. 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).

Summary/Conclusions: Status of c-mpl in ITP is ambiguous. Significant level of positivity in patient group who did not have splenectomy might be 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 preeclampsia, 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 hematology 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 84-105]. 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).

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Bleeding complication has been collected in order to know if there is related to%IPF.
Summary/Conclusions: Thrombocytopenia is a potential risk of bleeding during the labor. A high ITP indicates either consumptive or recovering thrombo-cytopenic disorders, such as immune thrombocytopenic purpura, while low ITP 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 platelet count cut off level was lower (100 x10^9/L) than previously reported parameters (mAbs). The level of platelet count cut off level of 100 x10^9/L might be an independent bleeding factor which can be useful for detecting high risk pregnant patients. It should be corroborated in further studies.

Background: Chronic primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by enhanced clearance of platelet and impaired platelet production. Corticosteroid is the ministry line of treatment of ITP, patients who fail to response to steroid (steroid resistant) or who relapse (steroid dependant) face the options of treatment with second line including anti CD-20 monoclonal antibody rituximab. Rituximab is a chimeric IgG1 mono-clonal antibody. Rituximab is active in the antibody-dependent cellular cytotoxicity (ADCC), ADCC effectiveness is influ-enced by process of activation of effector cells via their immunoglobulin G frag-ment C receptors (FcRy). Fc receptors show distinct affinity to bind to IgG subtype specificities. Differential response to rituximab has been reported to correlate with specific polymorphism of two of Fcγ receptors: FcγRy (H131R) and FcγRy (V158F) in some diseases. 

Aims: To clarify the effect of FcγRy-131 R/H and FcγRy-158 V/F genes polymorphism on the response to rituximab in ITP patients.

Methods: We studied the frequency of the FcγRy (H131R) and FcγRy (V158F) gene polymorphism in, 100 chronic ITP patients; divided into 2 equal groups, first group received rituximab (375 mg/m2 dose weekly for four weeks) and the other group received non-mab therapy second line therapy. A polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP] was used to detect FcγRy-131 R/H and FcγRy-158 V/F genes polymorphism. Evaluation of platelet counts was assessed initially before starting second line therapy then weekly for 3 months. At the end of third month the response to second line therapy was considered according to the following criteria; complete response (CR) PLT >100x10^9/L, partial Response (R), PLT>30-100x10^9/L, no response (NR), PLT<30x10^9/L.

Results: Regarding FcγRIIa polymorphism, in the 100 patients; 28 patients (28%) had wild HH genotype, 41 patients (41%) have het-erogenous genotype (HR) and 31 patients (31%) have homozygous mutant geno-type (RR). In our study, the 100 ITP patients included showed wild type of FcγRy (V158F) gene polymorphism. By the end of month 3 of the second line therapy, only 3/18 patients (16.7%) carried FcγRy HH genotype. However it was not statistically significant. Among the 13 patients who achieved NR, lowest rate was patients carried FcγRy RR genotype (23.1%) compared to HR (38.5%) and HH (38.5%) genotypes. However it is not statisti-cally significant. The mean value of platelet count at end of week 1, Week 2 and Week 3 of rituximab therapy show statistically significant differences (P value 0.001) being higher in patients achieved CR than who achieved PR or NR.

Summary/Conclusions: The higher platelet count achieved early (end of week 1, 2, and 3) after rituximab is suggestive for a better response later (at end of M3). FcγRy RR genotype is predictive for better response to rituximab in ITP patients.

PB2114

IMMUNE THROMBOCYTOPENIA, EGYPTIAN EXPERIENCE WITH STUDY OF IL-17,GFβ, 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 thrombocyto-penia (ITP) is heterogeneous and complex.It has been studied in active and reactive ITP but not to same extend in chronic and persistent type. 

Aims: 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 TGF-β by ELISA to assess role of subtypes of T cells in the pathophysiology of ITP.

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 by combined skin and mucous bleeding (11.1%). Comparison between the cases studied and control groups revealed statistically significant lower platelet count 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 important. All cases were treated with immunosuppression medication. In ad-dition to another immunosuppression medication. No correlation between measured cytokines and platelet count. 

Summary/Conclusions: The higher expression of IL-12 and IL-35 is due to persistently higher TH1 activity which explain continuity of the disease while low level of IL-17 and IL-35 is due to low production of Th17 cells. The use of anticoagulant drugs 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.
PB2117

ASSESSMENT OF PLATELET REACTIVITY TO ASPRIN AND CLOPIDOGREL WITH POINT-OF-CARE VERIFINNOW® ASSAY AND TWO ALTERNATIVE METHODS IN PATIENTS WITH CEREBRAL ANEURYSMS TREATED WITH ENDOVASCULAR PROCEDURES

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Background: Stent thrombosis and hemorrhage are the main complications after endovascular procedures for cerebral aneurysm treatment. Identifying an optimal pre-procedure response to antiplatelet therapy is essential to guarantee a successful result. A high variability in the individual responses to the anti-gregent effect of aspirin and, specially, with clopidogrel has been reported. The VerifyNow® System (Accutronics, San Diego, CA, USA) performs a turbidimetric-based optical detection of induced platelet aggregation in response to major antiplatelet agents (P2Y12 inhibitors, aspirin, GP IIb/IIIa inhibitors).

Aims: 1) To measure the antiplatelet effect of aspirin and clopidogrel with the point-of-care VerifyNow® assay in patients with brain aneurysms before undergoing endovascular treatment. 2) To compare the results with two alternative methods: impedance aggregometry, and PFA-100.

Methods: 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 clopidogrel at a dose of 75 mg daily 7 to 10 days before testing aspirin and clopidogrel 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 responders to aspirin and clopidogrel respectively. PRU <85 was considered hyper-response to clopidogrel. 2) Impedance aggregometry from whole blood (Multiplate® 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 ≥U and aggregation with ADP ≥47 U were considered good responses to aspirin and clopidogrel 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 ticlopidine, we performed it to analyze which hyper-responders to clopidogrel 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®, Multiplate® and PFA-100 respectively. Good response to clopidogrel was detected in 86.84%, 38.88% and 62.5% of the patients using VerifyNow®, Multiplate® and PFA-100 respectively. VerifyNow® identified 6 (13.78%) aspirin-resistant patients. However, PFA-100 and Multiplate® only showed a significant aspirin-mediated platelet dysfunction in 5 of them. Low response to clopidogrel was detected by VerifyNow® in 5 (13.15%) patients consistent with Multiplate® results. VerifyNow® identified 10 patients with excessive response, but only 2 of these results were reproduced by Multiplate® or COL/ADP. Multiplate® detected 19 patients (50%) with suboptimal response to clopidogrel, although these results did not correlate with those obtained by VerifyNow®.

Table 1.

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. Multiplate® assay using only
Methods: JAK2V617F mutation.

Aims: to DNA damage. Hematopoietic stem cells of JAK2V617F positive murine models associated with an increased level of reactive oxygen species (ROS) which also leads to healthy controls. Eleven patients had JAK2V617F mutation and twelve were JAK2V617F mutation negative. Significantly higher ROS levels were found in JAK2V617F mutation positive patients compared to JAK2V617F mutation negative patients.

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.

THROMBOPOIETIN-RECEPTOR AGONISTS IN ITP - EXPERIENCE OF A CENTER

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 adult patients, but only eltrombopag was 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. Patients 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-three (88.8%) patients were treated with eltrombopag (5 pediatric cases): 27 patients (81.8%) responded while 6 patients had resistance or disease progression to acute myeloid leukemia.

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, they are an urgent need for prospective studies to define the optimum 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.
Background: Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by immune-mediated platelet destruction and suppressed platelet production. ITP may occur concurrently or precede the occurrence of SLE, which would have great diagnostic significance. ITP may also be the first early sign of the disease. Few studies have addressed the risk of systemic lupus erythematosus (SLE) after ITP.

Aims: To estimate the risk of SLE after ITP in adult Jordanian patients.

Methods: All patients diagnosed with ITP and with a platelet count <100x10^9/L, between September 2002 and January 2017 were included in the study. Patients were retrospectively reviewed for diagnosis of SLE, and inclusion criteria included only those patients who had initial ANA screen at the time of the presentation of ITP. All patients with the diagnosis of SLE at the time and before the presentation of primary ITP were excluded from the study.

Results: This study included a total of 58 patients (43 females and 15 males) who were followed up for a period of 14 years. Their age at the baseline ranged from 16 to 65 years with a mean (SD) of 31.2 (13.3). ANA was positive in 11 (19.0%) patients. Over the period of follow up, 9 (15.5%) patients developed lupus. The incidence was 13.3% among males and 16.3% among females, with no significant difference (p-value=0.786). There was significant association between ANA and lupus in both genders. Only one patient with negative ANA and 81.8% of patients with positive ANA developed lupus (P<0.005).

Summary/Conclusions: SLE developed in patients with primary ITP in with 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 primary ITP diagnosis with positive ANA titer is of great importance as the risk of SLE is significant.

PB2122
TREATMENT OF REFRACTORY IMMUNE THROMBOCYTOPENIA WITH THROMBOPOIETIN RECEPTOR AGONISTS: OUR EXPERIENCE IN CHILDHOOD
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Background: Immune thrombocytopenia (ITP) is an autoimmune disease in which antibodies develop against platelets (pls) and dysregulation of cellular immunity result in premature destruction of pls 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 of all ITP patients eventually develop refractory ITP (RITP). Thrombopoietin receptor agonists (TPO-RA) stimulate thrombopoiesis and are an alternative

Aims: We present 3 different children with RITP treated with TPO-RA.

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 (Ig) and anti-D Ig. Rtx was started. After the 3rd dose, she responded temporarily along with fever, renal insufficiency and arterial hypertension, probably related to Ig A deficiency, not previously diagnosed. Romiplostim was indicated, reaching complete remission after 2 doses and it was stopped after the 4th dose, without any adverse reaction. Nowadays, plt count remains within normal limits (Figure 1A). CASE 2. A 5-year-old boy was diagnosed of ITP with cutaneous bleeding. He received treatment with prednisone and Ig with short response. Rtx was indicated; after 4th dose, severe thrombocytopenia and cutaneous bleeding persisted. Eltrombopag was started with response after 6 weeks of treatment (Figure 1B) and bleeding symptoms recovery. CASE 3. A 4-years-old boy with RTX was referred to our hospital. We decided to initiate treatment with Eltrombopag. He developed response after 4 weeks of treatment reaching 75mg/24h. Six weeks later, he presented 600,000/plts/L, so the drug was stopped. We observed a quick descent in plt levels and Eltrombopag was restarted with progressive response (Figure 1C).

Results: In all cases, splenectomy was avoided due to long-term risk of sep, as well as immunosuppressive agents like RTX in 3rd case. In 1st case, TPO-RA was able to stop with sustained response as described in some publications.

Summary/Conclusions: In our experience, TPO-RA appear to be efficacy and well tolerated in children.

PB2123
INVESTIGATION OF PLATELET FUNCTIONS IN PSEUDOTHROMBOCYTOPENIA
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Background: Pseudothrombocytopenia (pseudTCP), is incorrectly detection of low platelet counts in automatic blood counter devices and is most frequently caused by ethylene diamine tetra-aseticid (EDTA) induced platelet clumping and in vitro agglutination. Therefore, pseudTCP which accounts 15-30 of thrombocytopenic admissions, actually is not associated with a bleeding tendency. Causes of situations may be detected with a careful investigation of peripheral blood smears (PBS) by experienced clinicians but in centers which does not have these facilities; misleading of worried patients through advanced centers or even unnecessary treatments with steroids and platelet transfusions often occur.

Aims: In theory, formation of platelet clusters in the presence of EDTA requires functional adhesion molecules, so platelet adhesion and aggregation tests are expected to be in normal range. We aimed to investigate the capacity of simple platelet function analyzers for making the distinction between pseudo TCP and real thrombocytopenia.

Methods: Platelet functions were measured as collagen-ADP and collagen-epinephrine closure times (ColADP and ColEPI) by Platelet Function Analyzer (PFA-200™) for all patients who are referred to our clinic as thrombocytopenia (TCP, plt <150 x10^9/L) and value of this new method for determining pseu-

dTCP is compared with PBS which is accepted as the gold standard by using Receiver Operating Characteristic (ROC) curve analysis. PFA-200 system closure time is expected to be longer in true thrombocytopenia and normal in pseudTCP, but there is no study investigated this system for this purpose. Descriptive analyses were presented using means zstandard deviations for normally distributed variables or median and interquartile range (IQR) for nonparametric continuous variables. An overall p-value of less than 0.05 was considered to show a statistically significant result. This study is supported by Duzce University with project number of 2015.04.03.370 and these are pre-

liminary results.

Results: We included 59 patients who were referred to our clinic with thrombocytopenia (TCP, Plt<150 x10^9/L) and 11 healthy controls (Plt>150 x103/µL). Median age was 54 (IQR:37-68) for thrombocytopenic subjects and 37 (%63) of them were female. Median Plt count was 61 x10^9/L (IQR:30-90) in TCP group but WBC and Hb were not different from control subjects. Subjects referred with TCP were grouped with PBS as pseudo-

test.

TCP and real-TCP. There was no difference in terms of Plt, MPV, PCT, WBC or Hb between these groups but age was younger (median age 46 vs 62, p<0.05) and PDW was higher in pseudTCP group (med 17.6 vs 16.8, p<0.01). ColEPI and ColADP measures were significantly lower (med 125 vs 287 for ColEPI, med 84 vs 224 for ColADP, p<0.001 for both) at pseudTCP group. The capacity of ColEPI and ColADP values in predicting pseudTCP were analyzed using ROC curve analysis. We found that, when the manufacturer’s recommended cut-off value (150 s) was used, the sensi-
vability and specificity were 74.4% and 95%, with overall accuracy of 81.4% for ColEPI (AUC 0.913, SD:0.013, %95CI: 0.869-0.953). Similarly sensitivity and specificity were 79.5%, and 95%, with overall accuracy of 84.7% for ColADP using manufacturer’s cut-off value of 100 s (AUC 0.878, SD:0.055, p<0.001, %95CI: 0.770-0.986).

Summary/Conclusions: We concluded that, running PFA tests for everybody with thrombocytopenic counts, could be used for differentiating pseudo TCP and realTCP in centers which does not have conditions for proper BS. Especially long closure times excludes pseudTCP with a high specificity and could make clinicians quick decisions for further investigations.
# PB2124

**MANAGEMENT OF ADULT CHRONIC IMMUNE THROMBOCYTOPENIA. SINGLE CENTER EXPERIENCE**

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**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%), azathoprine (12%) rituximab and 8%: Rituximab. 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:** A total 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), cyclosporine 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=11), 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 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/µL (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 natural 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 eventuality 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.
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) supplement- ed 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 11.9). 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 need 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 sig-
nificantly 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=128), Non-Hodgkin’s lymphoma in III/IV st. (n=40) and chronic lympho-
cytic 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 well-being (PW), social/family well-being (SW/FW), emotional well-being (EW), functional well-being (FW), anemia-specific questionnaire – Anemia subscale (AnS), measuring fatigue-associated items – Fatigue subscale (FS) and non-fatigue-associated items – Non-Fatigue subscale (NFS).

Results: In the first group of patients (n=34) with severe anemia grade 4 QoL was revealed too poor; number of points in the subscale of PW was 14.0±0.9, in SW/FW – 14.2±0.7, in EW – 14.2±0.6, in FW – 18.5±1.3, in AnS – 34.6±2.2, in FS – 27.8±1.3, in NFS – 13.4±0.6. In the second group of patients (n=53) with anemia grade 3 QoL was poor too; in PW was 13.3±0.8, in SW/FW – 14.4±0.6, in EW – 9.9±0.7, in FW – 18.2±0.6, in AnS – 38.5±2.3, in FS – 26.8±1.7, in NFS – 12.0±0.7.

In the third group of patients (n=72) with anemia grade 2 QoL in the subscale of PW was 11.5±0.7, in SW/FW – 14.0±0.5, in EW – 8.6±0.6, in FW – 16.9±0.5, in AnS – 36.1±1.9, in FS – 25.5±1.4, in NFS – 11.6±0.6. In the fourth group of patients (n=70) with anemia grade 1 QoL in the subscale of PW was 11.3±0.7, in SW/FW – 14.3±0.6, in EW – 8.4±0.8, in FW – 16.0±0.7, in AnS – 34.3±1.6, in FS – 23.7±1.6, in NFS – 10.9±0.7. In the fifth group of patients (n=41) with anemia grade 0 QoL in PW was 11.7±0.9, in SW/FW – 14.9±0.8, in EW – 7.6±0.6, in FW – 16.4±0.5, in AnS – 34.6±2.2, in FS – 23.7±1.6, in NFS – 10.9±0.7. In the sixth group of patients (n=56) without anemia QoL in the subscale of PW was 11.7±0.7, in SW/FW – 13.6±0.6, in EW – 6.4±0.5, in FW – 14.8±0.7, in AnS – 23.4±1.5, in FS – 14.9±1.0, in NFS – 8.4±0.6.

Summary/Conclusions: QoL was found too poor in patients with Hb <8.0 g/dl. QoL wasn’t satisfactory in patients with Hb 8.0-11.0 g/dl. But the QoL improvement were greater in patients with Hb levels >11.0-12.0 g/dl (p<0.05). These data suggest that early correct anemia with red blood cells transfusions and erythropoiesis-stimulating agents can improve QoL in a clinically meaningful way.
Summary/Conclusions: The exercise shows that using local input changes the risk assessment could potentially influence local evaluations related to the access for LMWH treatment for CAT. Ticagrazin was found to be a cost effective LMWH over VKA in 6 European Countries as well as in Canada, when local medication costs were used. This was in contrast to the conclusion in the US.

PB2129
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 treatments of hematological malignancies with chemotherapeutic agents and radiation therapy, conditioning therapy in stem cell transplants. Regularly occurs at 3 to 10 days after chemotherapy and about to 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.

Methods: A retrospective analysis of 77 adult patients: 17 with hematological treatments and 60 with alopecia stem cell transplantation. 17 were diagnosed and treated between January 2016 and December 2016 with various hematological malignances (5 AML, 2 ALL – 1 Ph positive, 2 blastic phases of CML, 3 AML, 3 ALL, 1 TLL, 5 LMNH, 1 CLL, 2 SAA, 2 CML, 1 mycosis) with 10 cases of mucositis grade 3-4.

The conditioning regimen was mieloablative (14 cases) and reduced intensity (21 cases). The therapy was curative (10 patients) or palliative (17 patients) with curative intention for 10 patients (one was initially treated with curative intention and after that with palliative treatment). In 60 patients, GelX® was prescribed for treating grade 3-4 mucositis for the 10 patients (one was initially treated with curative intention and after that with palliative treatment).

Summary/Conclusions: 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.

Results: 60 patients allotransplanted, 30 patients experienced grade 3 and 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.

PB2130
EUROBLOODNET: THE EUROPEAN REFERENCE NETWORK IN RARE HEMATOLOGICAL DISEASES
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Background: Nearly all hematological disorders are rare diseases, affecting less than 1 in 2000 individuals, justifying their inclusion in a European Reference Network (ERN). ERN are networks created following the Directive 2011/24/EU on cross border health care which include nationally recognized Centers of Expertise aiming to ensure the same level of access to health services of all citizens. The ERN in Rare Hematological Diseases (RHD), results from a joint effort of the European Network on Rare and Congenital Anaemias (ENERCA), the European Hematology Association (EHA), and European hematology patient organisations represented in both the EURORDIS European Patient Advocacy Groups (ePAGS) and the EHA Patient Organisations Workgroup. EuroBloodNet gathers 66 highly skilled and multidisciplinary healthcare teams in 15 Member States, and advanced specialized medical equipment and infrastructures which will facilitate concentration of resources for the design, validation and implementation of high-quality and cost-effective services aimed at facing the challenges of RHD.

methods: EuroBloodNet’s mission is to improve the healthcare and quality of life of patients with a RHD by 1) Improving equal access to highly specialized healthcare delivery for RHD across Europe 2) Promoting best practices in prevention, diagnosis and safe clinical care across Europe 3) Disseminating cutting-edge knowledge and facilitating continuous medical education in the field of RHD. Providing access to orphan medicinal products and innovative drugs and safe exchange of clinical information 5) Fostering European cooperation in highly specialized procedures for diagnosis, promotion of clinical trials and innovative treatments and research.

Results: RHD are covered in two main thematic groups: non-malignant dis-orders include 4 sub-thematic areas: 1) Rare red blood cell defects 2) Bone marrow failure (BMF) and hematopoietic disorders 3) Rare Bleeding-Coagulation disorders and related diseases and 4) Haemochromatosis and hereditary iron metabolism disorders. Malignant diseases include 2 sub-thematic areas: 1) Myeloid malignancies and 2) Lymphoid malignancies. Methods and tasks aiming to achieve EuroBloodNet specific objectives have been split into five categories of Transversal Field of action (TFA): 1) Cross border health 2) Best practices 3) Continuing medical education 4) Telemedicine 5) Clinical trials and research.

Summary/Conclusions: EuroBloodNet, with the experience gained thanks to the EU-funded ENERCA and EHA, will seek to improve access to healthcare for RHD patients, to promote guidelines and best practice, to improve training and knowledge sharing, to offer clinical advice where national expertise is scarce, and to increase the number of clinical trials in the field.

PB2131
2016 REVISION OF WHO CLASSIFICATION OF TUMOURS OF HAEMATOPOETIC AND LYMPHOID TISSUES: IMPACT ON INVESTIGATING PATIENTS WITH ISCHAEMIC STROKE
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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 could increase the incidence of sub-clinical or asymptomatic PV.

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 included in the Indo-US Stroke Registry 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 web-based electronic database. From January, 2012 to March, 2014, 2076 patients with new onset ischemic stroke were evaluated 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 61 (2.9%) after 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 ischaemic 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 polycythemias, p=0.000. Separate analyses by gender was not significant in females, P=0.5; but significant in males, p=0.000. There were additional 29 males 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 the costs to each patient (- 7000 per our 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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2University of Medicine and Pharmacy of Craiova, 3Hematology, Filantropia City Hospital, 4Pathophysiology, University of Medicine and Pharmacy of Craiova, Craiova, Romania

**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 or without chemotherapy regimen due to a series of factors like intensity and density of drug doses, overall survival and quality of life of these patients. Various pathophysiological mechanisms responsible for the development of anemia are depicted in literature: pro-inflammatory cytokines and hepcidin action on iron metabolism and erythropoiesis, bone marrow failure caused by chemotherapy or by malignant lymphoma cells, cytokinins secondary to chemotherapy, immune peripheral destruction of red blood cells, iron and folate deficiency due to chronic bleeding.

**Aims:** To evaluate the prevalence of anemic syndrome in patients with non-Hodgkin’s lymphomas and the pathophysiological mechanisms involved in the development of anemia, in this study group.

**Methods:** A retrospective study was conducted on 85 patients (informed consent obtained) with non-Hodgkin’s lymphoma, who were admitted to the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, in between 2013 and 2015, in order to evaluate the prevalence and pathophysiological mechanisms involved in the development of anemia in this study group.

**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 with MALT lymphomas in 28%. NHL relapse on stage of disease were revealed: type I – 2.35%, 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 (47.25%), 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 groups. 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 led to an anemic syndrome in 18.05% of cases, whereas hemolysis of autoimmune cause was present in 9.7% of cases. In 12% of cases, diagnosed as malignant oridiopathic anemia, 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.

**PB2134**

**DEPRESSION AS THE PRESENTING SYMPTOM OF CENTRAL NERVOUS SYSTEM LYMPHOMAS IN NORTHWESTERN TURKEY**

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**Background:** Primary central nervous system lymphoma (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 onset or severe constitutional illness, with weight loss, sweats, and chills, are indicative of autoimmune diseases were reported. Presenting symptoms may include focal neurologic deficits, neuropsychiatric symptoms, signs of increased intracranial pressure, seizures or oculomotor palsies. Neuropsychiatric symptoms like depression, apathy, psychosis, confusional state, agitation, anxiety, or memory impairment were identified. 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-hemorrhagic 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 of 40 patients with CNS lymphomas were evaluated in a retrospective 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

**PB2133**

**TREATMENT IN ARGENTINA. SAFETY OF RITUXIMAB BIOSIMILAR (NOVEX®) IN THE ROUTINE USE**

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**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®. As part of the 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 as a part of real life 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 requested to fill a form indicating age and gender, treatment start date, treated pathological indication 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 Individual Case Safety Report. Occurrence rates were the Risk Management Plan (RMP) criteria. 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 restricted to 9 cases (3% of serious). The most frequent AR was cardiovascular manifestations (2 arrhythmia, 1 cardiac failure and 1 ischemic stroke), infections (1 pneumonia, 1 progressive multifocal leukoencephalopathy), neurologic (1 paresthesia), cytopoenias (1 pancytopenia) and cutaneous (1 bullous dermatitis). Conclusion:** The activities developed under this active pharmacovigilance 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 treatment. 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 biologicals, especially biosimilars, as a tool to assist in the knowledge about their safety profile.
from their medical files, type and treatment of lymphoma and survival were recorded. OECD international health 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 male (65%). Mean age was 60.5 years (range 38-78). Seven 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 neuropsychiatric symptoms while 16 patients presented with headache (40%) and 20 patients (50%) presented with neurological defects. 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.

**Summary/Conclusions:** OECD Health at a Glance data revealed that in 2013, the defined dose per 1000 per day is 35, range of Europe is 21-88. According to our data of Ministry of Health, use of antidepressants in the general population is 10.52%, mostly in women. Within these patients, 42.37% were anxiety disorders and 22.99% were depression. In the last five years’ statistics, 30% of antidepressants was prescribed for an antidepressant. The major group of physicians prescribing these medications was family and general physicians (45%). The most striking finding of our study was the majority of male patients receiving antidepressants before the diagnosis of CNS lymphoma with a mean delay of diagnosis as 2.6 months (0-10 months). Depression and anxiety disorders are the leading causes of disability and the importance of organic and underlying conditions should not be underestimated relying on the increasing need of antidepressants.

PB2136

**IMPACT OF U.S. FDA APPROVAL OF LENALIDOMIDE MAINTENANCE THERAPY IN THE FIRST-LINE TREATMENT OF MULTIPLE MYELOMA AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANT ON TOTAL HEALTHCARE COSTS**

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**Background:** Lenalidomide maintenance therapy after autologous hematopoietic stem cell transplant (auto-HSCT) in the first-line treatment has been shown to improve progression-free survival (PFS) and overall survival (OS) in multiple myeloma (MM) patients.

**Aims:** This study assessed the budget impact of the United States (U.S.) Food and Drug Administration (FDA) approval of lenalidomide maintenance therapy on total healthcare costs of a U.S. health plan.

**Methods:** An economic model was developed to estimate the incremental (additional) total plan costs (in 2016 USD) of maintenance therapy in each year for the first 3 years after lenalidomide monotherapy (R) maintenance therapy approval. The number of post auto-HSCT adult MM pts eligible for initiating maintenance therapy was estimated from published epidemiological data and an analysis of Connect® MM Registry data. Clinical endpoints for R-maintenance, including time on treatment, PFS and OS, were obtained from a meta-analysis of published clinical trials (CALGB, IFM, and GIMEMA). The use of common off-label maintenance therapies was considered. Types of costs included in the model were drug, drug administration, adverse events (AE), AE monitoring, one-time progression and terminal care costs.

**Results:** In a hypothetical U.S. health plan with 1 million members, the number of adult MM pts eligible to initiate post-asCT maintenance therapy was estimated to be 28. Among them, 14.8 pts initiated R-maintenance in Year 1, 15.2 in Year 2, and 15.3 in Year 3, representing an incremental increase of 2.9%, 4.2% and 4.4% after R-maintenance therapy approval, respectively. After considering additional costs of maintenance, as well as potential offsets resulting from delayed progression the incremental total healthcare costs by year are listed in the Table 1. Results were consistent across all total plan, per patient per year, and per member per month costs. Deterministic sensitivity analysis showed that the model results were robust to the variations of key model inputs.

**Summary/Conclusions:** Approval of lenalidomide monotherapy for maintenance after auto-HSCT in the first-line treatment of MM has minimal impact on total plan costs, primarily due to the small incident population and the already common use of lenalidomide in post auto-HSCT maintenance.

PB2137

**LAPAROSCOPIC APPROACH CAN EXTEND THE INDICATIONS OF SPLENECTOMY: ANALYSIS OF 31 CONSECUTIVE PATIENTS WITH MALIGNANT HEMOPATHIES**

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**Background:** Surgical resection of large spleens may eliminate a significant amount of tumor, allow definite diagnosis of malignant disorder, ameliorate abdominal symptoms and resolve cytopenia. However, because of short term perioperative events (25%) and long term immunosuppression (increased risk of infections caused by encapsulated bacteria) physicians can be reluctant to choose splenectomy, especially in older patients or patients with comorbidities. 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.

**Aims:** 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 them was used in 12 patients whereas in the remaining 38 cases, a semi-lateral position was chosen. All the patients received the triple vaccination (Streptococcus pneumoniae, type B Haemophilus influenzae, and Neisseria meningitidis). Patients characteristics, safety data such as early (<30 days) and late (>30 days) morbidities and mortality and efficacy (hematological recovery, accuracy of histological diagnosis) were analyzed.

**Results:** 19 patients underwent splenectomy for benign hemopathies (SBH) and 31 patients for malignant hemopathies (SMH). Non-Hodgkin’s lymphomas (12) and idiopathic myelofibrosis (10) were the most common causes of splenomegaly followed by chronic lymphocytic leukemia (7), hairy cell leukemia (1) and Hodgkin’s lymphoma (1). Patients’ age (67 +/- 12, years ranging from 36 to 87 in SHM, and from 11 to 71 in SBH), prior abdominal surgery (18/31) and spleen volume (1515 +/- 662 mL, ranging from 220 to 3000 mL in SMH, and from 90 to 1500 mL in SBH) were significantly higher in the SHM group (p < 0.05). There was no significant difference in surgical time (150 vs 146 min, p=0.8), blood losses (243 vs 402 mL, p=0.26) and duration of hospitalization (5.4 vs 7.5 days, p=0.19) between SMH and SBH. No case of locoregional dissemination was experienced. The early morbidity of the SHM group was 10% and 13% for the SBH group (p=1). Late morbidity was 0% in the SBH group and 13% in the SHM group (p=0.29). This could be explained by a combination of underlying disease and immunosuppression (2 sepsis and 2 deep vein thrombosis). There was one conversion to open surgery and perioperative mortality in each group (p=1). There was no significant difference in efficacy of splenectomy, with respectively 83% and 79% (p=0.91) or quality of histological sample for pathological report (88% and 85% respectively) for SMH and SBH groups. Out of 31 patients received a pre-surgical corticosteroid treatment, with a pre-surgical platelets level of 156 +/- 108 x 10^9/mL, white blood cell level of 15696 +/- 18950/mL and Hemoglobin level of 10.1 +/- 1.6 g/dL. Regarding the efficacy of LS in correcting hypersplenism in the SMH, a significant difference in term of platelets recovery after 1 month from the surgery was shown in patients efficiently Vs inefficiently operated (respectively 387 +/- 125 Vs 138 +/- 90 x 10^9/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 67 yrs) 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.

PB2138

**ARE WE AWARE OF ANXIETY AND DEPRESSION IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LEUKEMIA?**

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**Background:** Acute leukemia poses a high risk of stress for the patient during the process of diagnosis. The process after the diagnosis is challenging for the
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 its 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: 1. 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-traumatic issues. 3. Fear of isolation. 4. The issue of relationships. 5. Mind and body. 6. Children. 7. Faith. 8. Closure

As cited by S,A, a 49 year old man “I’m afraid to give in and die, help me to stay alive. And if I die, I want to know that I have left no unfinished business.”

Summary/Conclusions: From the therapy sessions it appears that the central issue is the patients learning to cope with the private space and the help seeking with it. 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 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.

PB2139

A ROOM OF MY OWN

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Results: The questionnaires were evaluated prospectively and the patients were divided into a control group, moderate anxiety group, severe anxiety group and depression group. The mean age of the patients was 38 years(range 17-72). The patients were divided into four anxiety groups. Levels of anxiety were 19 points or less were divided into no anxiety group, 20-29 points were divided into mild anxiety group, 30-39 points into moderate anxiety group and 40 points or more into severe anxiety group.

Summary/Conclusions: The rates of anxiety and depression were always higher in women. If we compared patients with different diseases, women were more anxious than men with the same disease.

Aims: 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-traumatic issues. 3. Fear of isolation. 4. The issue of relationships. 5. Mind and body. 6. Children. 7. Faith. 8. Closure

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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 disease. The aim was to study the mechanism of hydroxyurea inhibition of erythroid differentiation by exploring NO synthase (NOS) dependence.

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 erythroleukemic 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 ester hydrochloride. The same, but less prominent reduction 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 by 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 and 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 erythroleukemic cells, while phosphorylation of eNOS and activation of AKT/mTOR signaling was not blocked by phosphatidylinositol 3-kinase inhibition.

Summary/Conclusions: NO produg hydroxyurea demonstrated NOS dependence in inhibition of myeloid / erythroid differentiation, not influencing the hematological parameters.

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.

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, 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,SD) aged 6–17 years. 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 did not find significant differences in mean spirometry indices between the SCA cohort from London and northeast Italy (Table 1). Nevertheless, while an obstructive spirometric 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 ITA</th>
<th>Diff between means (5% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.9±4.7</td>
<td>11.3±2.5</td>
<td>0.6±0.1 ±0.4</td>
</tr>
<tr>
<td>Height (z-score)</td>
<td>-0.11±1.23</td>
<td>-0.28±1.09</td>
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<tr>
<td>BMI (z-score)</td>
<td>-0.55±0.55</td>
<td>-0.55±0.13</td>
<td>0.04±0.64 ±0.42</td>
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<td>FEV1 (mL)</td>
<td>-1.10±1.04</td>
<td>-2.80±0.97</td>
<td>0.04±0.72 ±0.66</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>-0.68±1.03</td>
<td>-2.80±0.43</td>
<td>0.04±0.24 ±0.74</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.

Summary/Conclusions: 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 erythroleukemic cell line.
Methods: The study is 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/gir1 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 alla gen deletion, and 1 (0.48%) glucose 6 phosphate dehydrogenase deficiency. No patient had congenital thrombotic diathesis. Eighteen patients (8.65%) had human leucocyte antigen (HLA) identical siblings. Hydroxurea 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 126 patients (60%). Change of exchange or transfusions existed in 25 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 13.87 years of age (median 6.77 years). Ten patients remained on immunosuppression, and 1 attained a marrow reversion. 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 (6.77%).

Summary/Conclusions: Early diagnosis like universal neonatal screening allows an effective health education, and antibiotic and osteopenia prophylaxis with 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. The efficacy of complement inhibition by eculizumab in the modified Ham test. Mixing eculizumab serum (ECU) with complement activated sera demonstrated a dose-killing relationship that was consistent across the 4 patients.

Figure 1.
Summary/Conclusions: Our results suggest that complement dysregulation is evident in asymptomatic SCD patients with increased Hbs levels, an important tool in everyday clinical practice. 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 of immigrant families 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, Eilthorn test, PMA spatial relations subscale). All tests were administered in Italian. A composite index considering parental education and employment was used to match socially, demographically and linguistically similar children. Group differences 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 two 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 associated to visible lesions in Broca’s area. In fact only 9 patients presented Silent Infarcts 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 (Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests. P-values: Visuo-spatial intelligence: not significant; Boston naming: ns; Phonol-Fluency: 0.004; Semantic fluency: ns.

Summary/Conclusions: Selective language problems may occur in children with SCD in the absence of clear neurological damage to language areas. These problems are explained by the executive dysfunction of patients with SCD and not by environmental factors like bilingualism. Cognitive rehabilitation or extra tuition may aid in overcoming these difficulties.

PB2146
UNDERSTANDING MEDICAL HISTORY, LIFESTYLE AND NEEDS FOR FUTURE THERAPIES FOR PEOPLE LIVING WITH SICKLE CELL DISEASE - IMPLICATIONS FROM A PATIENT SURVEY
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Background: Sickle Cell Disease (SCD) is an inherited blood disorder affecting millions of people. Sevuparin/DF02 is being developed to treat people suffering from SCD and is currently in clinical phase 2 for the treatment of the acute painful crisis in hospitalized SCD patients with intravenous infusion. This is called the Resolve program. In a second program called EASE, sevuparin/DF02 will be investigated as an on-demand treatment of early symptoms of painful sickle cell crises in an at-home setting via a subcutaneous injection. Searching in the literature and discussing with health care providers, it becomes clear that little is known about how the SCD patients sense these early symptoms of a painful crisis. In order to gain increased understanding of how people living with SCD experience daily life, coping with disease, support by health care providers and the demand for new therapies, a patient survey addressing these areas was conducted.

Aims: The aim with this survey was to gain deeper understanding of different aspects of life with SCD by providing a channel for patients to air their own views. 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.

Table 1.

<table>
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<th>Number of responders</th>
<th>Age</th>
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<th>Male</th>
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<td>79.3±8.4</td>
<td>58.6±12.1</td>
<td>African American/African descent</td>
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<tr>
<td></td>
<td>79.3±8.4</td>
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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 /BETA 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/beta thalassemia.

Aims: A prospective, long term evaluation of HU efficacy and safety in children and adolescents with sickle/beta 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: 0-3.2 vs median: 0.16 per study year after HU, 0.0-0.83, 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±1.9%, p=0.039), WBC count (9.56±3.67/μl vs 7.746±3.46/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 mild 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.
Background: Erythrocyte sickling and adhesion are favoured by cellular dehydration, which increases the rate of hemoglobin polymerization and cell sickling. Potassium ions activate transport and calcium-activated potassium channel (K+ channel) mediate erythrocyte dehydration in sickle cell disease and β-thalassemia. We investigated the in-vitro and in-vivo effects of various concentrations 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: This study was aimed at ascertain the efficacy of high potassium isotonic solutions in rehydrating sickle cell and possibly reversing the sickling phenomenon in vivo and in vitro situations.

Methods: Erythrocytes from twenty sickle cell anaemia (SCA) as well as 46 healthy subjects were studied. One part was treated with sodium metabisulphite (Na2S2O7) solution to induce maximum sickling while the other was subjected to different high concentrations of K+ in PSS as well as Cocos nucifera water (40mM, 80mM and CNw - 65mMOL/L) 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 40mK+PSS was not statistically significant. In both HB AA and SS subjects, MCHC was not significantly stable when compared to the pre-injection sample (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 of coconut water both in normal and SCA subjects. Another high potassium ion solutions can activate the rehydration of sickled 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.

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 prenatal diagnosis, 50% of Mairor 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 it is known that low levels of vitamin D 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.

Background: Sickle cell anaemia (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 in France 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 rate was 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”: 68% of patients had graduated from high school or achieved a higher level, 18% had graduated from professional education, 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 patients 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 interest on PE (52.1%) vs 31.3% that claimed to be 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 only help them understand the possible complications of their disease, but also allow us to adapt the long-term 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.
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: We reported 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 (IVIG) or erythropoietin. 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 transfusionally 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. Ht was 21.9%. WBC was 17.3 x 10^9/L and low MNHD (1200 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 RBCs and packed RBCs. Possibility of pre-existing vasculopathy mimics an acute vaso-occlusive 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 aetologies are useful. Whenever a DHTR is suspected, further RBC transfusion should be withheld unless absolutely 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. This present study 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.

PB2152

HBS MONITORING ON TOSOH G8 IN VARIANT HBA1C MODE IN CASE OF URGENT RCE

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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).

Methods: Within- and between-run imprecision were assessed using a sickle cell trait and a sickle cell anemia sample, aliquoted and stored at -80°C, twice using the variant HbA1c mode. Results were compared to our routine CZE Minicap Flex Piercing using 32 whole blood left-over HbS samples (HbS range: 9% - 93%). Data analysis was performed using Microsoft Excel Analyze-it version 0.998 (>0.95) and a slope and intercept of 0.94 (95%CI: 0.92 to 0.98) and linearity was observed. Passing-bablok regression analysis comparing TOSOH G8 and Minicap Flex Piercing showed an acceptable correlation coefficient of 0.998 (>0.95) and a slope and intercept of 0.94 (95%CI: 0.92 to 0.97) respectively. Differences in HbS results between TOSOH G8 and Minicap Flex Piercing ranged from -1.57% to +1.85% (mean difference: -0.05%). More specifically, for samples with a Hbs concentration <25%, Hbs results on TOSOH G8 differed between -0.34% to +0.36% compared to Minicap Flex Piercing. For samples with a Hbs concentration >25%, differences in Hbs results ranged from -8.76% to -0.43%.

Summary/Conclusions: In our clinical laboratory, TOSOH G8 is used in variant HbA1c mode to quantify HbA1c. Previous studies demonstrated reliable HbS identification using TOSOH G8 in variant HbA1c mode. Our study showed good analytical performance for HbS quantification using TOSOH G8. Good correlation with Minicap Flex Piercing system was found, although results were statistically not interchangeable. Our results suggest that TOSOH G8 in variant HbA1c mode generates lower HbS results in samples with a high Hbs concentration (>25%) compared to our routine analyzer. However, the goal of RCE is to achieve a post-transfusion HbS level of 30% or less. Therefore, results obtained with TOSOH G8 are clinically acceptable to monitor post-transfusion HbS levels. Importantly, HbS on TOSOH G8 can only be requested in case of urgent RCE. Our routine hemoglobinopathy screening will still be performed using CZE Minicap Flex Piercing in combination with CE-HPLC Variant 1TM.
Aims: Here we report our findings following a complete retrospective audit cycle, documenting the timeliness 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 respect 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.
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, PDWP

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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=60) 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 relapse-free survival (GRFS) were 45±2%, 43±2% and 35±2%, respectively. Cumulative incidence function (CIF) of neutrophil engraftment was 88.6% (85.9-91.4%). CIF for acute GVHD was 34% (30.1-38.4%) at 100 days. At 4 years chronic GVHD was 19.1% (15.7-23.3%), relapse incidence was 34.5% (30.1-38.8%) 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% (18.1-38%) 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% 1.07-2.14, p=0.02), and 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 ALLOGENIC STEM CELL TRANSPLANTATION

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Background: Allogeneic 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 except in case of BM from haploident donor haematopoietic progenitors (haploTPH).

Results: Thirty nine patients including 7 recipients with a history of SCT were enrolled. Median age was 61 (28-68), 21 were male, and 18 were female. Nineteen were acute myeloid leukemia, 11 myelodysplastic syndrome, 6 malignant lymphoma and 3 acute lymphoblastic leukemia. Donors were 4 matched related, BM, 5 -Ag/allele-mismatched unrelated BM, and 22 -Ag-mismatched CB. Thirty seven (94.9%) patients have passed 60-day post transplant. In 38 (97.4%) recipients, engraftment was obtained, a patient died before engraftment due to sepsis caused by enterococcus faecium (male CB recipient, 55y, day15). Median neutrophil recovery to over 500/µl was obtained on day 19 (16-38). Fourteen blood stream infections (13 bacteriaemia and 1 candidemia) judged as grade 3 toxicity and 2 cases (1 sepsis and 1 endocarditis) grade 4 toxicity were observed within 60 days post transplant. There were 2 deaths of post-engraftment due to cerebral bleeding (1 female CB recipient, 64y, day 46) and GVHD (1 male CB recipient, 60y, day 77) within 100 days. Although no relapse was observed up to day 60, 7 relapses were observed up to 1 year. Overall survival and disease-free survival were estimated to be 82.1% and 73.5% at 1 year post-transplant, respectively.

Summary/Conclusions: RIC using Flu/high dose AraC/Cy +/- TBI was well tolerated with acceptable low toxicities and was sufficient to allow donor cell-engraftment post allo-SCT for elderly or frail patients with hematologic malignancies. Longer follow up and another prospective study enrolling more patients regarding non-neoplastic complications. (See Figure 1).

Table. 1. Patient’s characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (%)</th>
<th>n (%)</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>51 - 60</td>
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<tr>
<td>61 - 70</td>
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<td></td>
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<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>15 (38.5%)</td>
<td>15</td>
<td>38.5%</td>
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<tr>
<td>Acute myeloid leukemia</td>
<td>7 (17.9%)</td>
<td>7</td>
<td>17.9%</td>
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<tr>
<td>Myelodysplastic syndrome</td>
<td>10 (25.6%)</td>
<td>10</td>
<td>25.6%</td>
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<tr>
<td>Malignant lymphoma</td>
<td>6 (15.4%)</td>
<td>6</td>
<td>15.4%</td>
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<tr>
<td>Acute lymphoblastic leukemia</td>
<td>3 (7.7%)</td>
<td>3</td>
<td>7.7%</td>
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<tr>
<td>Prognostic index (CI of PFLP)</td>
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<tr>
<td>Low risk: 23</td>
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<td></td>
<td></td>
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<tr>
<td>IntermEDIATE: 44</td>
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<tr>
<td>High risk: 85</td>
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<tr>
<td>Response pre-ASCT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Complete Remission:</td>
<td>70 (17.9%)</td>
<td>70</td>
<td>17.9%</td>
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<tr>
<td>Partial remission: 46</td>
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<tr>
<td>Stable disease: 1</td>
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</tr>
<tr>
<td>Number of Lines pre-ASCT</td>
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<tr>
<td>1 line: 1, 2 lines: 60</td>
<td></td>
<td>60</td>
<td>13.5%</td>
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<tr>
<td>3 lines: 1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4 lines: 7</td>
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<td></td>
</tr>
<tr>
<td>Conditioning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAM</td>
<td>85 (21.7%)</td>
<td>85</td>
<td>21.7%</td>
</tr>
<tr>
<td>Flu</td>
<td>21 (5.4%)</td>
<td>21</td>
<td>5.4%</td>
</tr>
<tr>
<td>TBI</td>
<td>30 (7.7%)</td>
<td>30</td>
<td>7.7%</td>
</tr>
<tr>
<td>Flu + TBI</td>
<td>6 (1.5%)</td>
<td>6</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

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.

PB2160

THE MANAGEMENT OF RELAPSED HODGKIN’S LYMPHOMA AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION: DONNOR LYMPHOCYTE INFUSION AND BRENTUXIMAB

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Background: Hodgkin’s lymphoma, is an heterogeneous malignancy wich is possible to cure. For those patients who relapse, chemotherapy followed by an autologous stem cell transplantation (ASCT) or haploTPH becomes a treatment option. Allogeneic transplantation (allo-SCT) is used for patients in relapse after auto-SCT or those with refractory advanced disease. Since 2012, with the experience of the Baltimore group, our Center has chosen the haploidentical family donor as a source for alloSCT in Hodgkin’s disease. Despite the promising results, the rate of relapse is between 25 and 35%, and there is not standardized treatment for this situation.

Aims: To analyze the outcome of post-transplant relapse treatment of haploident donor haematopoietic progenitors (haploTPH).

Methods: 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 88.3% (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, oropharngeal 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 second neoplasias (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 myocardopathy) 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).
Methods: We studied 127 adult patients who underwent ASCT following LEED or MCEC as the conditioning regimen against chemo-sensitive ML at four institutions in Japan between 1997 and 2015. Any type of pathological diagnosis was considered. The LEED regimen consisted of 140 mg/m² L-PM (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 -1). The MCEC regimen consisted of 200 mg/m² MCNU (days -8 and -3), 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 categorical variables. OS rates were estimated by the Kaplan-Meier method and comparing the log-rank test. Cumulative incidences of TRM and RRD were estimated by competing risk analysis. Values of p < 0.05 were considered significant.

Results: Of the 127 patients, 76 were male and 51 were female, and the median age was 32 years (range: 21-60). Underlying diseases were DLBCL in 74 patients, mantle cell lymphoma in 16, other B-cell lymphoma in 14, Hodgkin lymphoma in 9, and T-NK-cell lymphoma in 14. The disease status at the time of transplant was first complete remission (CR) in 68, advanced CR in 27, and partial remission in 32. As the conditioning regimens before ASCT, 81 patients (64%) received the LEED regimen and 46 (36%) received the MCEC regimen. No significant differences in patient characteristics, disease features, or transplant procedures were present between the two groups except for the following three factors: (1) ASCT in the later period (2007-2015) in the LEED group compared with the MCEC group (72% vs 13%; p < 0.01); (2) more frequent administration of rituximab before ASCT in the LEED group (84% vs 59%; p < 0.01); and (3) less frequent radiation therapy before ASCT in the LEED group (17% vs 37%; p = 0.02). The 5-year OS rates were not significantly different between the LEED and MCEC groups (77% vs 88%; p = 0.35). Likewise, both the 5-year CI of relapse and NRM were similar in the two groups (10% vs 13%; p = 0.61, NRM: 1% vs 8%; p = 0.71). In multivariate analysis that included the transplant periods, rituximab administration, and radiation therapy as independent variables, two or more prior regimens was extracted as an independent unfavorable prognostic factor for OS, but not conditioning regimens. Regimen-related toxicities within 100 days after ASCT were not significantly different between the LEED and MCEC groups. The 5-year CI of secondary MDS/AML were similar between the two groups (4% vs 3%; p = 0.62).

Conclusions: Our findings demonstrated that both the LEED and MCEC regimens showed sufficient anti-lymphoma effect as conditioning regimens before ASCT, with a 5-year OS rate of more than 70% in patients with chemo-sensitive ML. However, the LEED regimen is considered more preferable in comparison with the MCEC regimen based on the low frequency of severe regimen-related toxicities. A large-scale prospective study is warranted to confirm these findings.
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 (LVFS) 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.
expensive instrumentation, high reagent costs, and poor reproducibility between technicians and laboratories.

**Aims:** We developed and assessed an instrument performance of a newly-developed image-based microscopic cell counter (ADAM II™) for enumeration of CD34+ cell and its viability.

**Methods:** We developed and assessed an instrument performance of a newly-developed image-based microscopic cell counter (ADAM II™) for enumeration of CD34+ cell and its viability.

**Results:** Each analysis used 10 aliquots from one sample to assess the reproducibility. The correlation coefficient (R²) of each analysis was between 0.99 and 0.99, indicating high reproducibility.

**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.

**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 hematopoietic cell transplantation (alloHCT) 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 haploidential donors received thymoglobulin (ATG) 5mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included cyclosporine – methotrexate in myeloablative and cyclosporine – mycophenolate mofetil in reduced intensity 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/week 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 (3), matched (8) or one locus mismatched (8) volunteer unrelated and haploidential (1) donors. 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 GrII, 7 with GrIII) received steroid-dependent or refractory aGVHD and 11 (8 with GrIII, 3 with GrIV) steroid-refractory. ATG was administered simultaneously with ECP initiation in 6 refractory patients that further developed EBV reactivation (p=0.032) treated pre-emptively with rituximab. ECP was commenced at day +51 for 16 (4-20) sessions. The majority of patients (18/20) presented partial (6), very good (9) or complete (5) response to ECP. 1-year overall survival (OS) was 53% and significantly increased in steroid-dependent patients (78% vs 36%, p=0.041). Resolution of acute GVHD (p=0.026) and steroid dependence (p=0.023) were associated with improved OS, irrespectively of other factors.

**Summary/Conclusions:** Our study supports that ECP should be considered early as a second-line treatment in steroid-dependent or refractory aGVHD, before irreversible end organ damage has been established. Optimal timing of intervention, frequency, duration and tapering schedule of ECP need to be investigated in future studies.

**PB2167**

**RAPID RECONSTITUTION OF NK1 CELLS IS ASSOCIATED WITH THE LOWER INCIDENCE OF GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENIC TRANSPLANTATION**

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**Background:** The balance between immunostimulation and immunoregulation in T cell immunity is achieved by a Th1/Th2/Th3/T1r 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 0.10, 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.
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 896 (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 (sever central nervous system disease). The overall survival rate was 55.1%, and 32 patients had died (cause of death: progressing 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 of 1.60 × 10^9/kg in the patients who achieved engraftment and 1.01 × 10^9/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 × 10^9/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 × 10^9/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 × 10^9/kg, the risk of graft failure is very low.

PB2170

COLONY FORMING CAPACITY OF HEMATOPOIETIC STEM CELLS MOBILIZED INTO PERIPHERAL BLOOD WITH VINORELBBINE AND CARFILSOMIB IN COMBINATION WITH STEROIDS

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Background: One of the alternative method to mobilize stem cells from bone marrow to peripheral blood is using vinorelbine with granulocyte colony stimulating factor (G-CSF). The specific features of vinorelbine are absence of hospitalization necessity and predictability of leukocytesphere's optimal time. But there is not enough data to conclude whether vinorelbine is safe for hematopoietic stem cells.

Aims: The aim of the study was to determine the colonyforming capacity of hematopoietic stem cells mobilized into peripheral blood with vinorelbine and G-CSF.

Methods: Data of 11 patients with multiple myeloma (MM) and 1 patient with Hodgkin lymphoma (HL) were analyzed. Vinorelbine was injected IV in dose 50-70 mg (35 mg/m²). Daily lenograstim dose was 10 μg/kg. The number of BFU-Hodgkin lymphoma (HL) were analyzed. Vinorelbine was injected IV in dose 50- carfilsomibe (1 patient) in combination with steroids. A patient with HL was haematopoietic stem cells mobilized into peripheral blood with vinirelbine and treatment included borteszomibe (11 patients), lenalidomide (5 patients) and there is not enough data to conclude whether vinorelbine is safe for haematopoietic stem cells transplantation necessity and predictability of leukocytapheresis' optimal time. But 1x10⁵ cells of leukocytapheresis product were set into Petri dish with MethoCult H 4435 full medium. Control group was consisted of hematopoietic stem cells donors’ data in which G-CSF monotherapy was used for mobilization.

Results: The median patients’ age was 55 (43-64) y. Induction courses for MM treatment included bortezomib (11 patients), lenalidomide (5 patients) and carfilsomibe in combination with dexamethasone. A patient with MM was treated with ABVD scheme. Leukocytespheres were started on 6-8 day, here-in with 9/11 (75%) patients on 7 day. The number of gained CD34+ was 1.7-7.8x10⁹/kg (Me 3.3x10⁹/kg). Median number of BFU-E, CFU-GM, CFU-GM and CFU-Macrophage in patients’ group was 207, 180, 14 and 9 accordingly.

The results were not significantly different from control group data: 168, 170, 10 and 12 accordingly; p<0.05.

Summary/Conclusions: We conclude that mobilization regimen with vinorel-bine in combination with G-CSF does not damage colonyforming capacity of hematopoietic stem cells.

PB2171

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 (AH SCT).

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 undergoing HSCT. 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, total protein and albumin were analyzed. Univariate, multivariate Cox regression models and Kaplan-Meier survival analyses were performed. The specific and sensitivity of graft association with disease-free survival (DFS) , over all survival (OS), early non relapse mortality (+30 day ) and late non relaps mortality (+100 day).

Results: Pretransplantation low uric acid levels were detected in 57% (±20.8) median UA levels and low UA levels were significantly associated with DFS (HR: 0.52; p = 0.027). None of the creatine, total protein and albümün were significantly asso-ciated with DFS (HR:0.98; p= 0.98, HR: 0.87 =0.60, HR: 1.15; p = 0.66 ). There was no significant association between UA, creatine, total protein and albümün levels and overall survival (HR: 0.84; p = 0.48, HR: 2.10; p = 0.057, 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 other 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 a antioxidant compound. Pretransplantation low urine acid (UA) levels are associated with worse disease-free survival and overall survival in patients who underwent allogeneic hematopoietic stem cell transplantation. 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 underlyng such mechanisms, such as reduced antioxidan-ty activity and high hyperuricemia (UA > 6 mg/dl) is warranted.

PB2172

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 allo-geneic hematopoietic stem cell transplantation (allo-HSCT) even when the prophyllactic 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 evaluate the outcomes following hematopoietic stem cell transplantation (HSCT).

Methods: Nineteen patients with HSV-1/2 viremia and fifty-seven patients with-out HSV-1/2 viremia which were selected using the case-pair method after hap-loidentical 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 cystis (HC) in patients with and without HSV-1/2 viremia were 77% and 94% (p=0.003), respectively.
Results: The median time to platelet engraftment was 21 days (range, 11–80 d) and the incidence of oral mucositis was 36% (p=0.000). In a multivariate analysis, HSV-1/2 viremia was associated with delayed platelet engraftment (p=0.038), a higher incidence of oral mucositis (p=0.000) and severe HC (p=0.038). However, HSV-1/2 viremia was not associated with non-relapse mortality (34% vs 31.5%, p=0.26), 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 LEUKEMIA OR MYELODYSPLASTIC 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 at 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 transplant.

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 16 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 included Flu (125mg/m²) combined with Mel (80mg/m²), n=21 or 140mg/m², n=105.
- Total body irradiation (TBI) (1.15Gy) was used in 86 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.
- A median follow-up of 46 months (range: 15-144 months) for the survivors, the 1-year GRFS and disease-free survival (DFS) and OS were 57%, 61% and 70%, respectively.

On univariate analysis for all patients, pre-transplant factors associated with a worse OS and DFS included BM/PB 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 1.5-4.6, p=0.001) and H/V (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 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, rDRI (HR 2.5, 95% CI 1.4-4.7, p=0.003) was adversely associated with OS, so were H/V 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 was 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 INCIDENT 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 haploidentical (haplo) HSCT, with an incidence of 10% to 70% and a high mortality 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.

Methods: We analyzed all consecutive haplo-HSCT from family donors performed at our Hospital between 2013 and 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 (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 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 received 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 immunosupression state of the patient, we also observed all these pts had positive serum viral load for CMV.

Figure 1.

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 I 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 development 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 hematological 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. For detailed result see Figure 1. 18 patients didn’t have any signs of relapse, graft failure or acute graft-versus-host disease at all observation time. In this group on day 30% of cells with donors genotype was - 97,17±0,75; on day 60 - 97,5±0,75; on day 90 - 97,65±0,75 (p<0,05). 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 126 days (range 62-268). 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 unit (Hebenstreit et al., Leuk Lymphoma 2014) showed OS 51% and NRM 30% at 4y. Ibrutinib appears to be a feasible option in a limited setting. Although further testing with larger numbers of patients is required.

Aims: To evaluate Th, Treg and bone marrow cell short-term chimerism after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Results: 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 old. In 6 patients 16, male=8. Myeloablative conditioning regimen was used for 11 patients. The other 13 patients underwent 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 chimerism. Graft rejection was evaluated at +30, +60, and 90-day in bone and blood marrow. Peripheral blood mononuclear cells (PBMC) were isolated using standard protocol. Cells were sequentially incubated with CD4-biotin and anti-biotin microbeads (Milteny Biotech, Germany). Next pure fraction of T cells (CD4+CD25high) was obtained by positive selection with the use of anti-CD25 microbeads. DNA was isolated by AmpliSens DNA-sorbB nucleic acid extraction kit. Chimerism was assessed by the STR-PCR analysis (polymerase chain reaction with a panel of primers for loci of short tandem repeats).

Background: The study sample consisted of 91 patients [mean age: 37.±13.5 years, n=52 (57%) males, n=39 (87%) autologous SCT, n=12 (13%) allogeneic SCT, median overall survival in months: 12 in males and 19 in females]. Death was observed in 6 (11.5%) males and 2 (4.2%) females. Patient characteristics were similar across gender categories except for weights (kg) and Body Mass Index (kg/m2): 88.1 and 28.6 vs 65.2 and 25.0, in males and females respectively (p<0.05). Changes from PET/CT scan to 3 months post transplantation were analyzed at the level of the L3 to calculate TAT, VAT and Waist Circumference (WC). Data was analyzed by gender since body composition parameters differed significantly between the two categories in the literature.

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class II haplotype groups connected with autoimmune diseases in Caucasian analysed. It was possible to categorise 15 out of 16 NRM patients into 5 HLA and antigen and sex mismatches, HLA patient/donor haplotypes were factors connected to NRM - age, comorbidity score, patient/donor HLA allelic microangiopathy (TAM) and infections - both viral and fungal. Additionally to GVHD 2-4 was diagnosed previously, accompanied by transplant associated complications with septic shock. In one patient - antiviral treatment refractory CMV elevated risk of death. Later two patients developed infectious bacterial com-

Antileukaemic treatment can be reason for higher treatment related toxicity and conditioning with cytreduction phase. Active disease and highly active conventional chemotherapy. These patients were subjected to sequential con-

in patients with relapsed/refractory acute leukaemia without remission after antileukaemic treatment. These patients were subjected to sequential con-

nonrelapse mortality (NRM) (17%). There were 9 early deaths (before day +100) - 6 cases with post-transplant AML relapse in 2nd CR. Out of 93 procedures of unrelated donor transplantation in four-year-period: 2012 to 2016 - 93 transplant procedures in 86 patients.

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-relat-
ed mortality (TRM)). We used SPSS V.23 to calculate the cumulative Mortality incidence by the KM test and the Cox proportional hazards model.

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 (3 patients), graft failure. Out of re-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 condi-
tioning 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 comp-
lications with septic shock. In one patient - antiviral treatment refractory CMV encephalitis with massive macroangiopathy 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 (3 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 haplo-
types in population, further analysis is required.

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 (3 patients), graft failure. Out of re-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 macroangiopathy 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 (3 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 haplo-
types in population, further analysis is required.

PB2179

HAPLOIDENTICAL STEM CELL TRANSPLANTATION WITH HIGH DOSE CYCLOPHOSPHAMIDE POST-TRANSPLANT IN HIGH RISK HEMATOLOGIC MALIGNACIES: RISK FACTOR AND OUTCOME ANALYSES IN OUR CENTER

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Background: Allogeneic 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 appli-
cation of allo-HSCT in hematological malignancies. Haploidentical HSCT (Hap-
lo-HSCT) offers the benefits of rapid and nearly universal donor availability and, in the past decade, has been accepted worldwide as an alternative treat-
ment 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 Hap-
lo-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-relat-
ed 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-
enue between OM from the different states of EBMT (p=0.356) and DRI (p=0.07), however we found a statistically significant difference for ECOG (p=0.028) (Figure 1b) and Sorror (p=0.016). On a pairwise analysis of the OM we found no statistically significance for EBMT, and found a statistically significant difference between the patients with low-high DRI (p=0.01), intermediate-high DRI (p=0.001), ECOG 1-0 (p=0.046) and Sorror 0-5 (p=0.003). The multivariate analysis showed that ECOG 2 vs 0 (p=0.013, HR=4.46, 95% CI: 1.69–11.47) and Sorror 2-3 vs 0-1 (p=0.041, HR=19.55) and Sorror 4-5 vs 0-1 (p=0.005, HR=282.48) were significantly related with a higher incidence of OM. Five patients died of infection (41.67%), 3 of disease progression (25%), 1 of relapse (8.33%) and 3 of other causes (25%). Six patients died of TRM (50%). The CI of TRM was 10.5% at 1 m and 31.6% at 3m, 6 m, 1, 2 and 3 years (Figure 1c). When we analyzed the TRM depending on the different status scores we only found a statistically significant difference between TRM incidence from the different states of ECOG (p=0.038) (Figure 1d) and no statistically significant difference for EBMT (p=0.386), DRI (p=0.372) and Sorror. The multivariate analysis also found statistically significant differences between ECOG 1-2 (p=0.018) and EBMT 1-5 (p=0.046), for Sorror we found a marginal statistical significnatification between 0-1 (p=0.052), 0-2 (p=0.052) and 0-5 (p=0.052), for DRI we found no statistically significant difference. On the multivariate analysis we found no statistically significant correlation between TRM and the physical status scores.

**Summary/Conclusions:** Despite the fact that Sorror, EBMT and DRI scores are widely validated to establish the risk of patients undergoing HSCT, in our experience ECOG remains a useful score for assessing the risk of TRM on patients receiving Haplo-HSCT. We think further studies with a larger sample would be necessary to confirm our results.

### PB2180

**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% (58.2–94.4), 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%). There was significant difference in the viability of CD34+ cells between the two groups (p=0.02, respectively). We analyzed 81 autologous PBSC transplants after stored at -80°C by using simplified method. We studied 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 30.3 (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:** The simplification of cryopreservation represents a means of holding a potential therapeutic modality in reserve for use at a future date. In this study, PBSCs can be safely stored for at least 36 months by a simplified method at -80°C. The loss of the viability of CD34+ cells was greater when the granulocyte content was over 10% than in cells with less than 10% of granulocytes. The effect of reduced CD34+ cells viability seems important for engraftment. Differences in the day to platelet >50x10^9/L between the two groups based on the granulocyte concentration (>10% concentration against <10% concentration) was observed. Thus, a lesser granulocyte content could give a more reliable graft with better quality. Further research is necessary to observe the effect of long-term cryopreservation period and granulocyte content on the viability of stored CD34+ cells.
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 Bortezomb-Based (Bor-) 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) have an 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 conserved candidacy to get Bor-Based conditioning. 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 III/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), progression (1). Median of days of disease (d) of disease before initiation of conditioning 3 of the patients (3) had PR as initial response. Median number of cycles administered: 6 (3-12). 3 of them didn’t responde. 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 other 3 patients are in treatment or preASCT evaluation. Characteristics of the after-rescue ASCT-patients. Median age at ASCT: 62 (49-70). All of them received Melphalan 200 mg/m2 as conditioning treatment.

Results: Morbidity or mortality (M&M) (0%) of ASCT procedure in refractory patients is similar to non-refractory patients. After a median follow up of 46 months from diagnosis for all ASCT-candidates group, the refractory patients get an overall survival of 46.2 months (3-72 m). Any of them have relapsed yet. 2 of them are in biological relapse without need of treatment. Summary/Conclusions: Patients refractoriness to induction may receive ASCT after a rescue treatment LenDex based, as is effective in this group considering the characteristics of the patients and the regimen. New combinations (tripllet) with new drugs with LenDex-based treatment may improve the responses rates and overall survival before and after of ASCT procedure in these group.

PB2183

SAFETY AND Efficacy of TBF CONDITIONING in patients UNDERGOING ALLOGENIC STEM CELL TRANSPLANTATION. A RETROSPECTIVE SINGLE CENTER EXPERIENCE.

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Background: The optimal intensity of myeloablation with a reduced-toxicity conditioning (RTChem) regimen to decrease relapse rate after allogeneic stem cell transplant (allo-SCT)without increasing non-relapse mortality (NRM), has not been well established.

Aims: To evaluate the prognostic effect of disease status before ASCT - complete remission (CR) - on survival (OS) and comparison between risk groups was performed by using the log-rank test. Univariate analysis was performed and significant predictors at the 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 HDT and ASCT between 2007 and 2016 in a single institute. All patients received peripheral blood cell support after conditioning with BEAM regimen (carmustine 300mg/m2, etoposide 800mg/m2, Ara-c 1600mg/m2 and melphalan 140mg/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.

Results: We included 83 NHL patients, mainly males (72.3%) with a median age at diagnosis of 51 years (18-65). The most prevalent histological subtypes were diffuse large B cell lymphoma (53.0%), mantle cell lymphoma (36.1%) and follicular lymphoma (10.7%). The median number of therapeutic lines was 2 (1-5). Patients with diffuse large B cell lymphoma and follicular lymphoma were mainly treated with R-ChOP/R-CVP (82.5%) at first-line. For those who did not achieve a CR or relapsed after first-line treatment, (R)-ESHAP/DHAP/ICE (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 (0.3-72.6) 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 ASCT, 4 patients were not assessed for response due to early death by toxicity. Of the remaining, 70 (88.6%) 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, 16-89), female to male ratio:5/16. The stages and MIPI scores at diagnosis were as follows: 5% stage II, 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-/ICE in 5 patients (24%), R-/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, 6-123 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 autologous hematopoietic cell transplantation as well as two patients underwent allogeneic HCT. All patients received intravenous and paracrine, secretome, and immunomodulatory effects of stem cells would appear to be the likely mechanisms of application for ASD therapeutics.
Aims: Evaluation the benefits of HSCT in patients with ASD.
Methods: We describe two cases of patients with ASD who underwent HSCT for acute lymphoblastic leukemia (ALL) and whose symptoms were markedly decreased like an improvement of social interaction, communication, and CR after ASCT.
Results: The first patient is an 11-year-old girl with ASD who was diagnosis with Ph-positive ALL in October 2011 (at the end of treatment, BCR-ABL remained positive). She underwent a matched sibling HSCT in March 2015. The conditioning regimen was total body irradiation (TBI) and cyclophosphamide. During the 20-month follow-up period, we observed improvement in social interaction, communication, and behaviours. According to The Childhood Autism Rating Scale – CARS, prior to HSCT, she had a score of 39 (Severe Symptoms of ASD Disorder), and she currently scores 30 (Mild-to-Moderate Symptoms of ASD). The second case is a 7-year-old boy with ASD, Asperger Syndrome, who was diagnosis with ALL in September 2012. He presented with bone marrow and testicular relapse in May 2015 and underwent a matched unrelated HSCT in November 2015. The conditioning regimen was Etoposide, ATG and TBI. During the 12-month follow-up period, we observed improvement in social interaction, communication, and behaviours. According to CARS, prior to HSCT he had a score of 36 (Severe Symptoms of ASD Disorder), and he currently scores 24 (Minimal-to-No Symptoms of ASD). There is no treatment for ASD thus every effort to minimize the symptoms are valuable. In both cases, social interaction was significantly increased, and the aggressive behaviors decreased. Clinical cases have reported responses in autistic children receiving HSCT [21].
Summary/Conclusions: Several incurable neurological disorders have shown benefits with cellular therapy. Thus, autism should be explored as an indication. Clinical studies are an immediate need to fully explore its potential in autism.

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 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 (JMLL) and 4 patients had myelodysplastic related acute myeloid leukemia (MDRAML). Diagnostic cytogenetics included monosomy 7 (n=2), trisomy 8 (n=3), KRAS (n=1) or normal/other (n=8). Patients received a median of 6.8x106 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 (MSD), five from a matched unrelated donor (MUD) and 4 from haploidentical donor. Conditioning regimen consisted of busulfan/cyclophosphamide in MDS/AML 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 JMLL (n=1), secondary MDS (n=1) and MDR-AML (n=1). Eleven patients received a median of 35.5 days post-transplant and two of them died. 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 behavior. 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 JMLL given HSCT. All children with MDS should be referred for allogeneic HSCT soon after diagnosis.
PB2188

RELATIONSHIP BETWEEN URIC ACID LEVELS AND CARDINAL FINDINGS IN A LARGE COHORT OF B-TALASSEMA 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. Multivariate regression analysis was performed on the resulting G', G'' and G* curves and Principal Components Analysis was used as display method. 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 2.7±2.7 mg/dL; P=0.0001). UA levels directly correlated with BMI (R=0.25, P=0.0003), 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±2.9 mg/dL, P=0.03). The multiple regression model identified BMI (T-value 3.7, P=0.0003), 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 T2* values (β=1.9, 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 B-TALASSEMA BY A CHEMOMETRIC APPROACH

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Background: Several reports showed 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, we have investigated the viscoelastic profiles of red blood cells from patients with β-thalassemia. The hemorheological profiles of blood samples obtained from healthy subjects and thalassemic patients were studied by chemometric tools in order to develop a model of prediction of TM patients.

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 carry out a clear distinction of thalassemic status. From this study, the results are that 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 THALASSEMAIA 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 causal agent is a RNA virus, transfused mainly through the fecal-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 among 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 21.6 (range 2-58 years). 42% were male and 58% female. 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 same patient population had been transfused with 3.384 blood units. The detection of HEV RNA was performed by Real-Time RT-PCR method (hepatitis2@ceerTools 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 titration 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.
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 (21.53±5.44). Blood samples from β-TM patients and healthy controls were analyzed for Pro12Ala gene polymorphism using polymerase chain reaction-restiction 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 was 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 homozygous 12Ala polymorphism. Both had normal blood mass index, lipid profile, ejection fraction and elevated serum ferritin (4923 ng/l in heterozygous patient and 4886 ng/l in homozygous patient). Ejection fraction was 70% in heterozygous patient and 68% in homozygous patient. Only one male control (10%) has homozygous 12Ala polymorphism (Table 1).

**Table 1.**

**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

**THALASSEMSIA 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 universal increase at a young age, into a chronic disease, with a constantly increasing life expectancy. This is attributed, amongst others, to the use of improved chelation therapy. Since prolongation of life expectancy has occurred only in recent years, there is little data regarding the older population with TM and TI.

**Aims:** 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) & 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=0.02 and 72.4 vs 84 fl, p=0.04, 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.

**PB2194**

**EVALUATION OF LIVER IRON CONCENTRATIONS IN CHILDREN WITH BETA THALASSEMIA INFECTED WITH HEPATITIS C VIRUS BEFORE AND AFTER SPIRULINA THERAPY BY MAGNETIC RESONANCE IMAGING**

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**Background:** Magnetic resonance imaging (MRI) assessment of liver iron concentration (LIC) is necessary for quantitative staging of iron overload in children with β-Thalassemia. There is no enough evidence about the effect of spirulina therapy on LIC.

**Aims:** To assess LIC by MRI in multitransfused β-thalassemia children infected with HCV before and after Spirulina Therapy.

**Methods:** Thirty multi-transfused β-thalassemia children infected with HCV were subjected to clinical evaluation, appropriate laboratory investigations and assessment of LIC by MRI. They were classified according to LIC into mild...
chelating agents. The efficacy of the treatment was estimated through MRI (Table 1).

1) treatment with one chelating agent did not succeed in decreasing heart and liver iron, 2) agranulocytosis or severe neutropenia due to deferiprone (DFP) can cause significant heart, liver and endocrine morbidity.

Methods:

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before starting DXF/DO combination therapy</th>
<th>Mean into the DXF/DO combination therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ferritin (meq)</td>
<td>1.36 (g/ml)</td>
<td>1.36 (g/ml)</td>
</tr>
<tr>
<td>Liver iron Concentration (LIC) (meq)</td>
<td>3.6 (mg/g)</td>
<td>3.6 (mg/g)</td>
</tr>
<tr>
<td>Cardiac T2* (ms)</td>
<td>24.1 (16.5-35.3</td>
<td>24.1 (16.5-35.3)</td>
</tr>
</tbody>
</table>

Discussion:

The combination treatment was well tolerated without adverse events or effects on liver and kidney function.

PB2196

EVALUATION OF THREE AUTOMATIC DEVICES FOR HEMOGLOBINOPATHY DIAGNOSTICS IN MULTI-ETHNIC POPULATIONS

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Background: We have tested three different dedicated haemoglobin separa-
tion devices for their capability of performing the diagnostics of hemo-
oglobinopathies. These involve the Variant II™ HPLC (BioRad), the Capillaries2 capillary electrophoresis (Sebia) and the most recently introduced HPLC, Pre-

1) treatment with one chelating agent did not succeed in decreasing heart and liver iron, 2) agranulocytosis or severe neutropenia due to deferiprone (DFP) can cause significant heart, liver and endocrine morbidity.

Methods:

Methods: 10 TDT patients were treated with a combination chelation therapy of DXF (50 ±10mg/kg/d) and DFO (44±12mg/kg/d for 2-6 days/wk in 12hr or 24hr infusion rates). Reasons for starting this combination treatment included: 1) treatment with one chelating agent did not succeed in decreasing heart and liver iron, 2) agranulocytosis or severe neutropenia due to deferiprone (DFP) treatment and 3) adverse events recorded with increased doses of one of the chelating agents. The efficacy of the treatment was estimated through MRI measurements of heart and liver iron (T2*mean, and LIC) in combination with serum ferritin levels. Liver enzymes (ALT, AST) and serum creatinine were used to monitor safety of the treatment.

Results: Five of the 10 patients had significant liver hemosiderosis (LIC >15 mg Fe/gr d.w.) and 3 had heart iron overload, of which one significant (T2* >1.9 msec) (Table 1).

Summary/Conclusions: Spirulina therapy may have favorable effects on lowering the values of LIC in children with β-Thalassemia infected with HCV.

PB2197

RED BLOOD CELL EXTENDED PARAMETERS IN HAEMOGLOBINOPATHIES

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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.

Aims: 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 thalassaemia (TDT) patients attending the Thalassaemia Unit in a tertiary hospital in Athens, Greece.

Methods: 89 haemoglobinopathie heterozygotes (32 men, median age of 29 years & 57 women, median age of 30 years) were included in the study and classified into three groups; group B: β-thalassaemia heterozygotes, N=46; group C: α-thalassaemia heterozygotes, N=21 and group D: Hb O-Arab heterozygotes, N=22. We retrospectively recorded the results of full blood count analysis on Sysmex® XE-5000 analyzer including%HYPO-Hb and%MicroR, to investigate their values in haemoglobinopathies and their correlation, if any, with Hb A2 levels in het-

erbocytes and Hb variants.

Methods: Reference ranges were obtained from 175 healthy adult subjects (27 men, median age of 34 years & 148 women, median age of 30 years); control group (group A), 89 haemoglobinopathie heterozygotes (32 men, medi-
an age of 29 years & 57 women, median age of 30 years) were included in the study and classified into three groups; group B: β-thalassaemia heterozygotes, N=46; group C: α-thalassaemia heterozygotes, N=21 and group D: Hb O-Arab heterozygotes, N=22. We retrospectively recorded the results of full blood count analysis on Sysmex® XE-5000 analyzer including%HYPO-Hb and%MicroR, of Hb pattern analysis (TOSOH®, G7) and ferritin levels (Roche®, cobas e411). All subjects included in the study presented ferritin levels within the normal range for age and gender. Statistical analysis: one-way ANOVA (Tukey post-
hoc), Mann-Whitney, Pearson’s correlation tests were applied. Reference ranges were calculated as the means±2SD of the distribution. P value <0.05 was considered to be statistically significant. Data refer as median (percentiles).

Results: 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 inde-
pendent of gender and age (P=0.715, P=0.168 & P=0.073, P=0.843). There was a statistically significant difference between the reference ranges calculated by one-way ANOVA for both parameters (all P <0.0001). Heterozygous β-thalas-
saemia presents statistically significantly higher%HYPO-He values [116.4 (4.2-
27.6)]% as compared to groups A [0.3 (0.2-0.3)]% & C [1.9 (0.6-4.6)]%, D [0.8 (0.4-
0.8)]% and D [0.8 (0.4-0.8)]% and D [0.8 (0.4-0.8)]% and D [0.8 (0.4-
0.8)]%. Heterozygous β-thalassaemia presents statistically significantly higher%MicroR values [41.5 (22.9-58.7)]% as compared to groups A [1.5 (1.1-2.0)]% & C [10.8 (7.9-20.5)]% and D
Background: Diagnosing α-thalassaemia requires second line diagnostics involving DNA analysis. Multiplex ligation probe amplification® (MLPA®) is a molecular technique introduced as a diagnostic tool for α-thalassaemia. This semi-quantitative technique determines the relative copy number of up to 60 DNA sequences and is able to detect deletions and duplications in a DNA sample. A novel commercial tool, the α-Globin StripAssay®, aims to detect the most common α-thalassaemia deletions and point mutations. The test involves three steps: DNA isolation, PCR reaction and a hybridization step to test strip containing allele-specific oligonucleotide probes immobilised as an array of parallel lines.

Aims: Our objective was to evaluate the α-Globin StripAssay® as a useful alternative for MLPA® in second line α-thalassaemia diagnostics.

Methods: Eight samples, including 7 known deletions (__SEA, __THAI, __MED, homozygous and heterozygous - α3.7, heterozygous - α2.8, (α20.5) and 1 mutation (Hb Constant Spring) were analysed using multiplex Gap-PCR (deletions) and Sanger sequencing (point mutation) at the Leiden University Medical Center. These samples were anonymised and analysed in duplicate by MLPA® and α-Globin StripAssay® at our center. A comparison of diagnostic performance, interpretation, turnaround time (TAT) and costs (reagent and labour) was done.

Results: There are no significant differences between the MLPA® and the α-Globin StripAssay® results and each identification corresponded to the result of the reference lab in Leiden. MLPA® however provided additional information about underlying polymorphisms. Interpretation of the α-Globin StripAssay® was easier and faster compared to MLPA®. The α-Globin StripAssay® proved to have a shorter TAT, but on the other hand, the costs for MLPA® were significantly less.

Summary/Conclusions: Despite its straightforward interpretation, shorter TAT and the possibility of detecting both (known) deletions and point mutations, the significantly higher costs of the α-Globin StripAssay® may hinder its routine use. Specialised laboratories are usually acquainted with the MLPA technique and in these settings the ability to detect both known and unknown deletions is a plus for research purposes.

Background: With the improvement in availability of blood transfusion practices and progress in chelation therapy, there is an increasing population of thalassemic patients surviving into adulthood in developing countries. However, there is scarcity of clinical, biochemical and radiological data showing cardiac and hepatic iron assessment in these chronically transfused individuals.

Aims: 1. Cardiac and hepatic iron assessment in young adults with TDT. 2. Compare the ferritin level with T2* MRI finding.

Methods: In this prospective observational study we analysed demographic details, clinical features and cardiac and liver iron assessment of young adults with (TDT) at recently established adult thalassemia clinic at PGIMER, Chandigarh, India. For cardiac and liver iron assessment serum ferritin, ECG, 2D Echo, MUGA scan, Liver function test, Fibroscan (if indicated) and T2* MRI of Liver and heart was done. All patients who were diagnosed in childhood and referred to adult haematology unit at age ≥18 years and had received more than 20 blood transfusions were included in the study.

Results: A total of 53 patients (n=53) were analysed. The mean age was 23 years. Majority of patients (56%) were male. The average age at diagnosis and at first transfusion was 7 months & 11months respectively. The average years of PRBC transfusion was 23 yrs. The average number of transfusion in last 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 ferritin level at the time of registration was 2019 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).

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.

Background: Diagnosis of thalassemia (Thal) in a Mediterranean country as Spain, could be thought as endemic, but few data are available so far. Moreover, attention to hemoglobinopathies is focused on sickle cell disease. Aims: The aim of our study is to find out the prevalence of Thal and clinical significant hemoglobinopathies other than sickle cell diseases in a referral center for newborn sickle screening, 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.

Methods: The study is observational, unincertic, descriptive and retrospective, carried out in December 2016 in a tertiary hospital in the Community of Madrid, Spain. All patients diagnosed with Thal and other not sickle-hemoglobinopathies who had attended at least once to the hematology clinic were included. Demographic characteristics (date of birth, gender, country of birth) and clinical ones (genotype or Thal type, 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 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 (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 Africa, 25% from Asia, and 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. Two out 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...
spleenectomy. None of these patients had sepsis or meningitis. Three Thal patients underwent allogeneic stem cell transplantations and then remain on complete chimerism in the present moment. 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 I. Tartaglione1 ,2, M. Caiazza3, A. Bonadies1, D. Roberti4, M. Casale1, S. Sciancuta1, F. Rossi5, S. Perrotta1

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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 without any 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 Thalassaemia (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 revision 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: 365 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%) Pretransfusion Hb was lower in patients with prolonged clotting times, demographics, disease history, comorbidities 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; the proportion of clotting test after blood transfusion could be advisable to overcome the low Hb effect.

PB2202

COMPOND HETEROGOUSITY FOR HAEMOGLOBIN ADANA AND α-THALASSAEMIA IN GREECE. CLINICAL PHENOTYPE AND GENETIC COUNSELING

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Background: Haemoglobin (Hb) Adana (HbAa2cQ.179A) in interaction with deletional and nondeletional α-thalassaemia mutations leads to HbH or, less commonly to thalassaemia intermedia with clinical manifestations varying from asymptomatic forms to severe anemia. First line screening tests are unable to detect the highly unstable variant. Aims: We report two cases of Hb Adana co-inheritance with the α-thalassaemia 3.7 kb deletion - the only α-thal and Hb Adana double heterozygosity cases diagnosed in subjects of Greek origin. Methods: The first case concerns a 3 year old girl, born from parents referred for genetic counseling at the 11th week of a second gestation. The mother showed an Hb of 10.7g/dl, MCV 80.7 fl, MCH 26.4 pg, Hb A2 2.8% and Hb F 1%, with positive inclusion bodies, and her ethnic (Greek) and regional background was of high risk for thalassaemia. The partner came from the same region, and he showed an Hb of 13.8g/dl, MCV 8.5 X 10\(^{12}\)/L, MCH 73.1 fl, MCHC 23.5 pg, Hb A2 2.4% and Hb F 2.3%, while her ferritin levels were 228ng/ml and inclusion bodies were found. On clinical examination she was found to be of normal weight and height for her age, but presented with paleness, icteric sclera and mild splenomegaly. Genetic analysis revealed that the carrier had the α-thalassaemia 3.7 kb deletion defect. The father carried the non deletional Hb Adana. As suspected from the hematological data, their offspring was a compound heterozygote for Hb Adana variant and a 3.7 kb α-thal deletion. The second case concerns an 11-year-old boy, diagnosed with Hb Adana co-inheritance with the α-thalassaemia 3.7 kb deletion at the age of 8 years. At diagnosis, findings were compatible with a very mild phenotype and growth was not impaired. The boy retained a mild hypochromic microcytic anemia (Hb=109g/dl, MCV 71 fl, MCH 23 pg, RDW 18.6%, reticulo 1.5%), until adolescence but at the age of 11 transfusion initiation was decided due to marked splenomegaly and limited weight and height gain. For the following years he was transfused approximately once a month, necessitating chelation therapy. Weight, height and pubertal development were normal by the age of 15, but splenomegaly persisted. Splenectomy was decided and transfusions were stopped shortly afterwards. During the following months the boy retained an Hb of 9.5 g/dl, however, he complained of constant fatigue and impaired physical activity and asked to get back on a transfusion program. Results: In both cases diagnosis was incidental highlighting the mild phenotype. However, the co inheritance of Hb Adana with the 3.7 kb α+ thal deletion is rare, with only the presenting cases in Greece, and in a few other families in Turkey, Southeast Asia, Philippines and Albania. The clinical phenotype of the combination seems to be a mild disease with a non-transfusion-dependent thalassaemia intermedia phenotype. Nonetheless, clinical severity prediction is always a difficult issue and phenotypes may change overtime as demonstrated by the second case described above.

Summary/Conclusions: Long follow-up of such rare cases is necessary in order to gain as much information as possible, so as to offer the best management to the patients and the most accurate genetic counseling.
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 v 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 weigh in PGPL-treated rats on 68.5% comparatively with control rats. The thrombus weigh 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 antplatelet activity in rats blood plasma. PGPL pretreatment lead to prevention of experimental venous thrombus formation. Therefore, PGPL may be used as 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, typical Haemolytic Uraemic Syndrome (HUS), Cancer associated TMA, Atypical HUS, Infections TMA, SLE and Transplant TMA.

Aims: This study is to focus on the treatment among the TMA and the outcome of the disease.

Methods: The data was collected from year 2012 to 2016 from Ampang Hospital via the electronic hospital information system (EHIS) and external records traced from Haemostasis labarory in Ampang Hospital as well as from other hospitals nationwide.

Results: There were total of 243 suspected TMA cases, encompassing 97 (39.9%) males and 146 (60.1%) females. The median age for this cohort was 34 years. Only 54 (24.15%) patients were diagnosed as TTP based on ADAMTS-13 activity ≤10%. Patients were treated by using complete case details from Ampang Hospital cohort (69 cases). From this cohort, only 59 cases had ADAMTS-13 activity testing. There were 24% Primary Acquired TTP, 5% typical HUS, 3.4% pregnancy TMA, 3.4% atypical HUS, 3.4% Pregnancy TMA, 20.3% Transplant TMA, 1.7% Cancer associated TMA and 37% TMA of other causes. The average plasma exchange was 8.4 cycles, and was higher in patients with ADAMTS-13 activity of ≤10% (11.4 cycles) as compared to those with ADAMTS-13 >10% (7.7 cycles). No infectious diseases were transmitted as a result of plasma exchange or plasma infusion. Treatments used in the patients included immunosuppressant therapy like cyclophosphamide (36.5%), monoclonal antibody like rituximab (36.2%), bortezomib (16.6%), cyclophosphamide (10.1%), cyclosporine (10.1%), and vincristine (26.1%). The survival outcome seemed to be worse among the patients with TMA in comparison to other groups (log-rank, p<0.0001). Transplantation was also associated with higher odd of death among TMA cases (OR: 14.8571, 95% CL: 1.7385, 126.9707). Those with confirmed TTP was inevitably doing better than the others in terms of overall survival (log-rank, p=0.0299). The odds of death was 4.36 times higher in patients with ADAMTS-13 activity >10% (OR: 4.36, 95% CL: 1.0961, 17.3714), indicating secondary TTP may have inferior treatment and disease outcomes than primary TTP like congenital or acquired TTP. Besides, the complications of the disease were also evaluated which revealed 26.9% of renal failure and 52.2% of neurological deficit. Furthermore, 8.7% were complicated by Venous 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%.

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.
Background: Antiphospholipid antibodies (APLS) have been implicated in vascular (atherosclerosis), venous (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 three; namely; T2DM, uncomplicated T2DM and healthy control. Each group had 70 subjects matched for sex and age. Lupus anticoagulant (LA) was assayed using DRVVT (technoclone 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 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 control subjects 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 to -3.0 to -2.1) and in uncomplicated T2DM and healthy control subjects (p<0.001, 95%-CI to -2.8 to -2.0) there was a significant difference in mean HbA1C between Ecarin clotting time (DTI test).

Conclusion: 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 THR789ALA GENETIC VARIANTS CORRELATE WITH DISEASE SYNDROME 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 Thr789Ala single nucleotide polymorphism is thought to affect factor level and function. Aims: This study aimed to investigate the prevalence of the vWF Thr789Ala 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 (RFLP-PCR) principles.

Results: vWF: Ag levels were significantly higher in MI (111.6±24.77 IU/dl) and UA (110.2±23.44 IU/ml) patients compared to healthy controls (71.1±23.72 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 Thr789Ala heterozygous and 12.9% were Thr789 homozygous. Regarding the MI group, Ala789 genotype was present in 34.6%, Thr789Ala genotype was the predominant genotype and was seen 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; 47.7% were Ala789 homozygous, 33.1% were heterozygous and 19.2% were Thr789Ala heterozygous and 12.9% 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 Thr789Ala genotype was shown as an independent risk factor of MI. Summary/Conclusions: The study shows that vWF Thr789Ala 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, large 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 enzymes or fibrinogenases because of their role in dissolving 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 substances, 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 by use of affinity chromatography on Blue-Sepharose and size exclusion chromatography on Superdex 75- PG. Platelet aggregation was determined by AT-02 aggregometer (Medtech, RF). The platelet count was adapted to 2.5x10^12 platelets/μl 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^-4 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 parameters 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 pharmacodynamics and studies for Dabigatran 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 (26%). We analyzed the values that increases the bleeding risk such as drug interactions, 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, a 7% with Rivaroxaban and a 3% with Apixaban. Thirty-five patients (15%) developed bleeding of which 11% had a minor bleed (Wu et al, 2014). In our series, in patients with dabigatran and who didn’t have therapeutic range of the drug, and measurement of serum creatinine, we didn’t found significant difference. However, the incidence of bleeding in patients with dabigatran and who suffered bleeding, we found a significant prolongation of aPTT and PT, demonstrating the importance of laboratory tests prior to the administration of these agents and in emergency situations, for these reasons should include PT measurement of serum creatinine.
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, Abakaliki, 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 were identified (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, 87 (16.2%) 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 of 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 prothrombin test 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 anticoagulation 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 anticoagulation 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.

PB2211

INTERLEUKIN -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 proinflammatory as compared with anti-inflammatory mediators. 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 proinflammatory mediator such as IL-1, IL-6, and IL-8 from monocytes/macrophages. Three important single nucleotide polymorphisms are the IL-10-1082 polymorphism, 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 -592C/A 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: In group A (provoked DVT), a higher prevalence of IL-10 -592C/A mutation was found compared to group B. The frequency of -592C/A mutation was 0.20 and 0.34 in group A and B respectively (P-value=0.037). A similar result was also found for the -1082AG mutation with a frequency of 0.20 and 0.22 in group A and B respectively (P-value=0.46). However, there is no correlation between two markers. No correlation was found between IL-10 -1082AG and IL-10 -592C/A mutation genotype distributions and VTE recurrence (P-value=0.94 and 0.36 respectively).

Summary/Conclusions: 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 (74.7%) have had radiological evidence of either 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 dysfibrinogenaemia(n=11) had the next high prevalence. One patient was diagnosed with Essential thrombocythaemia and one with SLE. None of the patients were positive for screening tests done for sickle cell disease and TINH.

Table 1.

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

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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. It may mimic vasculitis or sepsis, but has been reported in cases with severe thrombocytopenia and acquired prothrombin inhibitors. APS auto-antibodies are heterogeneous and may undergo post-translational modification during antigen stimulation altering its pathogenicity and thrombotic risk. Sepsis and associated disseminated intravascular coagulation is a known phenomenon where cytokines influence pro-coagulant and anti-coagulant pathways on multiple levels, induce haemostatic chaos.

Aims: Demonstrate the role of sepsis in triggering life threatening CAPS, and highlight the management strategies used in this highly complex and fatal disease.

Methods: Prospective case study illustrating two separate atypical CAPS 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 equilibrium was exacerbated by respiratory tract infection where she presented with severe headaches. Subsequent investigations demonstrated multiple atrialmatic intra-cranial haemorrhages followed by concurrent extensive cerebral venous thrombosis. 2nd episode (2017): She presented with subdural haemorrhage, preceded by fevers and respiratory symptoms. She then developed pleuritic chest pain and dyspepsia after temporary cessation of anticoagulation for 24 hours. Imaging confirmed multiple pulmonary emboli with areas of infarction. Respiratory symptoms worsened with progressive interstitial ground glass changes on CT consistent with atypical pulmonary infection. Shortly after low therapeutic anti-coagulation she developed acute abdominal pain and hypertension. CT showed significant bilateral adrenal haemorrhages. Management Strategies: (A) Rapid reduction in APS pathogenic auto-antibodies via plasma exchange, B cell depletion therapy and immuno modulation. (B) Treatment of underlying infectious trigger. (C) Judicious anticoagulation with anti-Xa monitoring and (D) long term hydroxychloroquine and statin therapy.

Results: The two life threatening presentations of CAPS were triggered by an infectious event, supporting the biological concept that anti-phospholipid antibodies can be immune modulated altering its pathogenic capabilities creating haemostatic havoc. There are similarities and a degree of overlap with sepsis and the pathophysiology behind disseminated intravascular coagulopathy. Rapid reduction in the pathogenic auto-antibodies using combination plasma exchange, immuno modulation and B cell depletion therapy is effective in this acute setting. Judicious anticoagulation and treatment of the precipitating infection is important in turning off the immune response driving this life-threatening condition.

Summary/Conclusions: CAPS is rare and life threatening, often triggered by an infectious event, trauma or temporary cessation of anticoagulation. It requires prompt recognition and timely commencement of therapy.
Summary/Conclusions: We concluded that intranasal administration of tripeptides Pro-Arg-Gly and Gly-Arg-Pro to organisms of healthy rats and in rats with experimental MS show antiplatelet and fibrinolytic effects of the blood. Thus, arginine-containing peptides could potentially be used as antithrombotic drugs that protect the organism from the blood coagulation and thrombus formation.

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 results are 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, Thrombinscope 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 24.86(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.

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 G.J. Ruiz Argüelles1,2, G.J. Ruiz-Delgado1, Y. Cantero-Fortiz2, M.A. Mendez-Huerta2, M. Leon-Gonzalez2, A.A. Leon-Peña4, A.K. Nuñez Cortés3, J.C. Olivares-Gazza4, J.A. Arizaga Barber4
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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 an incidence of 13% (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.
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 SECRECTIONS 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 secretions on hemostasis.

Methods: Adult specimens (both sexes) of Bombina bombina, Bombina variegata, Bufo bufo, and Butoles 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 lyophilized and kept at ~20 °C till use. In the experiments we used fresh prepared water solution of lyophilized skin secretions. Protein concentration was determined by Bradford method with BSA as a standard. Rabbit platelet-rich plasma (PRP), 2x10^6 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 MD 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 search for potential modulators of hemostatic system among the amphibian skin bioactive compounds. To establish their physiological and functional mechanisms of action, the further purification and characterization of components from the skin gland secretions are necessary.

PB2222

PLASMINOGEN-DEFICIENT PATIENTS

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Aims: Here we report our experience with local and intra venous fresh frozen plasma (FFP).

Methods: Our cohort consisted of 14 patients and their 8 first-degree relatives. The patients have been diagnosed between 3 months and 18 years of age, and the median age at the time of first clinical manifestation was 4.5 months (range 3 days to 12 months).

Results: Conjunctivitis is the main complaint, hydrocephalus and hearing loss follow. In 10 patients, lignonous membranes were surgically removed but all reoccurred. Nine patients were treated with intra venous and conjunctival FFP. Two patients had no complaints after treatment. Most patients needed transfusion with FFP every three weeks. Only one patient had severe endophthalmitis and local pressure eye before treatment. Two female patients and one male patient had undergone multiple surgeries for lignonous conjunctivitis despite being treated with FFP. The response rate to FFP treatment was 6/9 (66%). Another 8-year-old female with severe bronchial membranes was treated with FFP and t-PA through bronchoscopy. Venous thrombosis did not occur in any of the patients. Nine had consanguineous parents. The genetic evaluation of our patients revealed heterogenous mutations as well as polymorphisms.

Summary/Conclusions: The diagnosis and treatment of Plg deficiency is challenging, and there is no consensus on treatment. Topical and IV FFP may be used with clinical outcome.

PB2223

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 complete 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 abortion while 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 loss, only one case of preclampsia 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 Abruptio placentae and 5 cases with fetal growth restriction, 10 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 preclampsia.

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.

PB2224

LEARNING ABOUT VALIDATIONS OF THE DVT SCREENING TEST IN PATIENTS WITH SUSPECTED UPPER LIMB THROMBOSIS: A PERSPECTIVE FROM THE CLINICAL PRACTICE

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Background: Deep vein thrombosis (DVT) of the upper limbs represents 1-4% of DVT, most of them related to central venous catheter and/or malignancy. Thrombosis involving the deep veins (ie, subclavian, axillary, brachial) can lead to complications as pulmonary embolism (PE) and long-term sequelae. PE from upper extremity sources accounts for about 6% of cases. Initial treatment in acute context include fibrinolysis and subsequent anticoagulation (Grade 2C). When symptomsatology is mild and/or onset of symptoms undetermined (>2 weeks), minimum anticoagulation 3 months is recommended. If there are associated anatomical abnormalities, the possibility of surgical vascular thoracic decompression must be assessed.
Prevention of thrombus formation. Heparin: combination with adrenoceptor antagonists and PB2226

In the initial stages of fibrin formation, it causes the thrombus dissolution. Containing heparinoid contributes to the restoration of coagulation properties of blood clotting is installed in the experimental rats after application EP (recov-

Methods: The outcome 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 pre-

Methods: Experiments were carried out on 50 white laboratory rats weighing 200-230 g according to the ethical principles of the Helsinki Declaration. Anti-

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 lactifora roots (EA) on processes fibrin and thrombosis formation.

Methods: We used the standard coagulographic methods for determining anticoagulant activity by APTT test, antiplatelet, total fibrinolytic activity (TFA), fibrin-depolymerizing 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 prophylaxis and therapy by administration of subcutaneous 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-

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 (recovery of platelet aggregation to 98%, APTT -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 participate 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 antithrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.

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 pre-

Methods: Experiments were carried out on 50 white laboratory rats weighing 200-230 g according to the ethical principles of the Helsinki Declaration. Anti-

Summary/Conclusions: 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 pre-

PB2226

THE INFLUENCE OF HEPARINOID FROM THE PEONY Roots ON THE THROMBOSIS DISSOLUTION

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1M.V.Lomonosov Moscow State University, Moscow, Russian Federation

3Background: 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.

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Summary/Conclusions: Consequently, the extract of Paeonia lactiflora roots containing heparinoid contributes to the restoration of coagulation properties in blood of animals in antithrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.

PB2227

THE POLICY AND PRACTICE OF ANTICOAGULATION THERAPY AMONG CLINICIANS IN SOUTHEAST NIGERIA.

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Background: In the absence of anticoagulation therapy, the risk of Venous thromboembolism; deep-vein thrombosis (DVT) and pulmonary embolism (PE) in medically ill patients is comparable to that of 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 is 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 anticoagulant policy in the hospitals surveyed.

Methods: This is a multicentre cohort survey of the practice of anticoagulant therapy on blood coagulation during many years. The questionnaire was administered to clinicians in five tertiary hospitals in the southeast Nigeria. The questionnaire was designed to assess their practices anticoagulation therapy. 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 is 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 education and by the establishment of anticoagulation policies.

Transfusion medicine

PB2228

UMBILICAL CORD BLOOD PLASMA INFUSION PROMOTES BLOOD CELL RECOVERY IN INPATIENTS WITH ACUTE LEUKEMIA UNDERGOING CHEMOTHERAPY

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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 done every day until WBC >4×10^9/L and PLT >20×10^9/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 differences in age, gender and dose intensity of chemotherapy between the two groups (P>0.05). The average recovery time of the blood neutrophil granulocyte >0.5×10^9/L in experimental group and control group were respectively (6.5±3.26) days versus (12.92±4.75) days (P<0.05) and that of PLT >20×10^9/L was respectively (9.24±3.68) 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.

PB2229

TOWARD BETTER BLOOD TRANSFUSION PRACTICE: A SUCCESSFUL RED BLOOD CELL UTILIZATION TOOLS IN A TERTIARY CARE HOSPITAL

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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 at 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 was adopted by the American Association of Blood Banks and calculated for all various sub-specialities including Surgery, Internal Medicine, Pediatrics and Obstetrics and Gynecology. All procedures related to hospital transfusion practice were reviewed 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-specialities. 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 and reduce 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.
SAFETY AND EFFICACY OF A PROTHROMBIN COMPLEX CONCENTRATE IN VKA INVERSUAL AND OFF-LABEL INDICATIONS
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Background: Prothrombin complex concentrates (PCC) are highly purified mixtures of plasma coagulation factors that contain vitamin K dependent and anticoagulation 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 University 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 indication; 2) 33.33% in refractory coagulopathy in major bleeding (30 patients due to massive bleeding protocol activation, 43 patients in hepatopathy 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. 75% 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 invasive 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%. 83.26% 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.

TRACEABILITY OF RED BLOOD CELLS IN A HOSPITAL TRANSFUSION LABORATORY
M. Tserga1, A. Argyrou1,2, S. Nikolopoulou1, A. Gafou1

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.

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)

NON-HEMOLYTIC FEBRILE POST-PLATELET-TRANSFUSION REACTIONS IN HEMATOLOGICAL PATIENTS
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Background: Platelet concentrate (PC) transfusions are the main method of thrombocytopenia correction in hematological patients, but multiple transfusions could trigger alloimmunity and refractoriness to transfusions.

Aims: Comparison of post-transfusion reactions in hematological patients with individual matching and without individual matching receiving PC transfusion support.

Methods: In 2015-2016, we observed 948 hospitalized patients, who received 12,344 PC transfusions. Individual matching of PCs was performed by cross-matching on the Galileo-Neo (Immucor) analyzer. Statistical processing was performed using the chi-squared test with Yates’ correction.

Results: 107 of 948 patients developed refractoriness to PC transfusions (12% of total patients). Out of them, 21 patients received 389 PC transfusions without individual matching, 86 patients with individual matching with 1705 PC transfusions. During transfusions without individual matching to non-refractory patients, 0.003% of non-hemolytic febrile reactions (NHFR) have been record-

Background: According to European legislation (2002/98/EC, 2005/61/EC) as a requirement of hemovigilance system traceability (confirmation of final desitination 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.

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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>Transfusion</th>
<th>Post-transfusion reactions</th>
<th>% of all transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory before</td>
<td>21</td>
<td>39</td>
<td>19</td>
<td>0.02%</td>
</tr>
<tr>
<td>Refractory with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matching to refractory</td>
<td>206</td>
<td>372</td>
<td>196</td>
<td>0.02%</td>
</tr>
<tr>
<td>No-refractory without</td>
<td>21</td>
<td>39</td>
<td>19</td>
<td>0.02%</td>
</tr>
<tr>
<td>refractoriness matching</td>
<td></td>
<td></td>
<td></td>
<td></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, homozygous 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 undertake 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 occurred more than 3 years ago. 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.

Summary/Conclusions: The identiﬁcation of malaria antibodies is essential in SSA native donors and, by far, irreplaceable 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 wait some years for the forthcoming SSA second generation that will allow to fully match the entire SCD patient community.

REFERENCE

PB2234

EFFECTIVITY AND INFLUENCE OF IRON CHELATION THERAPY ON RED BLOOD CELL TRANSFUSIONS

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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 showedeficacy 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 poitransfused patients treated with Deferasirox in three counties Hematology Departments of North-West Romanian hospitals. Criteria of Deferasirox treatment: over 20 RBCT, serum ferritin level over 1000 microg/l. We created a data collection sheet including: demographic, information on patients’ disease, serum ferritin 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 ferritin level, number of RBCT before and after starting the treatment.

Results: We included 40 poitransfused 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 transfusions. The transfused 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 ferritin 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 diagnosed with low ferritin levels of ferrite, 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 discontinuing. In three cases, treatment was stopped because low ferritin level (under 500 microg/l). RBCT were administered before (mean 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 treat-ment 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. In both groups the difference of RBCT means (before and after the start of the treatment) are 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/medium 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 of achieving zero 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 lower to 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/L)</th>
<th>Hb Post (g/L)</th>
<th>yield-CH (g/awdl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>102</td>
<td>8.1</td>
<td>9.3</td>
<td>1.0</td>
</tr>
<tr>
<td>LT</td>
<td>97</td>
<td>7.4</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>
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Hb Pre: Pre-transfusion haemoglobin; Hb Post: Post-transfusion haemoglobin; PWC: Patients without post transfusion Hb level; 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. Aims: This study aimed 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 surface, core & x-regions).

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. There was a significant difference in HBsAb 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 favored 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 titers 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. According to 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 IgG and IgM titers in blood group A, anti-A IgG and IgM titers in blood group B and anti-A IgG and IgM titer in blood group O between males and females(p>0.05). However Anti-B IgG and IgM antibody titers 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.

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Summary/Conclusions: Female individuals of blood bank donors participated in our study have higher isohemagglutinin titer values rather than male individuals. Recurrent blood group incompatibility in pregnancy, invasive diagnostic and therapeutic approaches for risk analysis in fetal examination during pregnancy, perinatal complications causing fetomaternal hemorrhage after pregnancy or during birth and lastly autoimmune diseases cause the enhancement of isohemagglutinin titer values in female individuals. Regarding the gender differences; nutrition, vaccination and recurrent blood transfusion history of blood bank donors also effect and change the isohemagglutinin titers of individuals. Population specific isohemagglutinin titer values play a key role in blood donation policy of patients undergoing hematopoietic stem cell transplantation. Consequently; we predict that Turkish community-specific isohemagglutinin cut off titer values can be identified and we will hope our knowledge on this issue in the future with the increase of research is going to increase further.

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-

haematologica | 2017; 102(s2) | 881

Madrid, Spain, June 22 – 25, 2017
Thrombotic microangiopathies are a group of rare diseases characterized by non-immune microangiopathic hemolytic anemia, thrombocytopenia, 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.

Background: Thrombotic microangiopathies are a group of rare diseases characterized by non-immune microangiopathic hemolytic anemia, thrombocytopenia, 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.

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 (MCTD, 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 aproval of the drug of FVIII with specific activity 69.65±2.24 IU/mg protein.

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>S498</td>
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<td>E657</td>
</tr>
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<td>E836</td>
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<td>Kumar, S</td>
<td>E1397</td>
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<td>Kumar, S</td>
<td>P331</td>
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<td>Kumar, S</td>
<td>S135</td>
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<td>Kumar, S</td>
<td>E1361</td>
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<td>Kumar, S</td>
<td>S162</td>
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<td>Kumar, S</td>
<td>P325</td>
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<td>Kumar, S</td>
<td>E1225</td>
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<td>Kumar, S</td>
<td>P365</td>
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<td>Kumar, S</td>
<td>E1505</td>
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<td>S408</td>
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<td>E1225</td>
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<td>Kuykendall, A</td>
<td>P608</td>
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<td>Kadam, A</td>
<td>P375</td>
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<td>Kadam, A</td>
<td>S412</td>
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<td>S412</td>
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</tbody>
</table>
Lee, J, P333, E984, PB1993
Lee, J, P310, E850
Lee, J, PB1702
Lee, J, P259
Lee, J, SY, S491
Lee, J-H, E909, E984, E1177, PB1684
Lee, J-J, S459, S491, E1089, E1091
Lee, J-O, PB1993
Lee, J, P569
Lee, J, P292
Lee, J-Y, PB2165
Lee, J, E1373
Lee, J-I, E892
Lee, J, S498, P742, E984, E1513
Lee, J-W, P551, P561, P638
Lee, J-H, E1177
Lee, K-O, E979
Lee, K S, PB1747
Lee, K-H, E909, E1177
Lee, K, PB2055
Lee, K, E1608
Lee, L, E1208
Lee, M, S422
Lee, M, S147
Lee, M-N, PB2055
Lee, M-Y, PB2165
Lee, N, E852
Lee, N-S, E1608
Lee, O-L, PB1981
Lee, S, P635
Lee, S, PB1673
Lee, S-C, E1608
Lee, S, E912
Lee, S, S491
Lee, S, E1605
Lee, S, PB2055
Lee, S, E947
Lee, W-S, P259, P659, E1054, E1069, E1070, PB1673, PB1684, PB1993
Lee, W-M, E871
Lee, Y, PB1857, PB1993
Lee, Y-H, PB1850, PB2165
Leeds, J, S773
Leekstera, A, S461
Lees, M, P278
Lefebvre, C, S427
Lefebvre, C, E1001, E1178, E1361
Lefebvre, T, S453, P631
Lefebvre, G, PB2083
Lefort, S, E1458
Lefere, F, P631
Legdeur, M-C, S112
Legrand, A, P274
Legros, L, E856
Leguay, T, E856, PB1629
Lehali, R, P159
Lehman, J, E1502
Lehmann, L, P740, P748, E926, E1532, E1554
Lehne, M, PB1633
Lehner-Graiwer, J, P620
Lei, B, S103
Leiba, M, S101, P334
Leiblein, S, PB2175
Leich, E, PB1934
Leij, E, S425
Leiss, Z, PB1654
Leitao, S, PB1960
Leite, J, S135
Leitegb, A, PB2146
Leivas, A, P509, E1234, PB1933
Lekakis, L, S466, P522
Lekovic, D, E1316, E1319, E1328, PB1831
Leleu, X, P323, P345, PB1863, PB1987
Leleveld, D, E901
Lemaire, P, E1361
Lemeire, C, E1549
Leone, G, PB2162
Leone, S, PB1707
Leone, V, PB2056
Leonetti Crescenzi, S, E1340
Leong, T, S, PB2016
Leon, E, P336
Leon Gonzalez, M, E1500, PB2219
Leon Peña, A, E1500, PB2219
Leoncin, M, E1254, E1357, E1397
Leoncini, P, P, E917, E1167, PB1665
Leone, G, PB2162
Leone, S, PB1707
Leone, V, PB2056
Leo, M, PB1899
Leoni, P, P636, E1136, PB1954
Leontopoulou, K, PB2166
Lepkov, S, P220, E1133
Leporace, A, P616, PB1696
Leppa, S, S776
Lepretre, S, E856
Leroy, H, PB1629
Lesokhin, A, E1252
Lessene, G, P175
Lessi, F, E930, PB1697, PB1728
Lesty, C, E1001, E1361
Leval, A, S472, P508
Leuchter, N, E992
Leung, A, P167
Leung, K, P167
Leung, K, PB1730
Leung, M, S477, P517
Leung, N, P331, P673, E1240
Leung, T, P167
Leung, W, P509
Leux, C, P217
Levali, M, S110, P560, E940
Leval, A, S472, P508
Leuchter, N, E992
Leung, A, P167
Leung, K, P167
Leung, K, PB1730
Leung, M, S477, P517
Leung, N, P331, P673, E1240
Leung, T, P167
Leung, W, P509
Leux, C, P217
Levati, G, E1030
Levato, L, P605, E1021, PB1818
Leverger, G, S427, P716, E1349
Leveron, J, S460, P189, P675
Levesque, H, PB2150
Leveta, G, PB2195
Levin, M-D, S501, P340, P677, PB1798
Levine, B, S477
Levine, J, S477, P517
Levis, A, P667
Levis, M, S110, P560, E940
Levitas, A, E1073
Levy, A, PB1987
Levy, M, S793, P201, E1464
Lewandowski, K, P611
Leighton, A, PB1980
Leys, R, S501
Lhermitte, V, E856
Lhermitte, L, E1236, E1386
Lhospice, F, E1386
Li, A, P405
Li, B, E1330
Li, C, E856
Li, C, PB1941
Li, C, P167
Li, C, P662
Li, D, P663
| Niederwieser, C, E927 |
| Niederwieser, D, P388 |
| Niederwieser, D, P261, P380, P555, E1506, PB1819, PB2175 |
| Niedoszytko, M, P709 |
| Nielsen, A, E1267, PB1995 |
| Nielsen, T, S774, S775 |
| Niemann, C, S109 |
| Niemeyer, C, P350, E1167 |
| Niemöller, C, P656 |
| Niepel, D, PB2009 |
| Niesiobedzka-Krezel, J, P594 |
| Niesvizky, R, S408, S458, E1244 |
| Nieters, A, P656 |
| Nieto, J, E1444 |
| Nieto, W, P584, E1019 |
| Nieto, Y, E1538 |
| Nieto Vazquez, A, PB1891 |
| Nifenecker, M, E923 |
| Nigauri, C, E962 |
| Niggli, F, S480 |
| Nigita, G, E917 |
| Niioka, T, E1053, PB1999 |
| Nijland, M, P567 |
| Nijs, G, S141 |
| Nikita, M, E862 |
| Nikoš, V, E970, PB1870 |
| Nikolopoulou, S, PB2231 |
| Nikolov, O, E1471 |
| Nikolova, Z, S467 |
| Nikolovski, S, S443 |
| Nikulina, E, E1409 |
| Nikulina, O, E1161 |
| Nislan, B, E1826 |
| Nimr, M, E930 |
| Ninnagui, C, E962 |
| Nishida, H, E1229 |
| Nishida, K, S124, P299 |
| Nishikawa, E, P171 |
| Nishimura, J-I, P578 |
| Nishio, K, E1590 |
| Nishio, N, P171 |
| Nishi, K, E1230, PB2158 |
| Nisoli, J, S788 |
| Nitigl, C, S469 |
| Nicol, V, E970, PB1708 |
| Nikolopoulou, S, PB2231 |
| Nikolov, O, E1471 |
| Nikolova, Z, S467 |
| Nikolovski, S, S443 |
| Nikulina, E, E1409 |
| Nikulina, O, E1161 |
| Nilsson, B, E826 |
| Nilsson, L, S805 |
| Nimmagadda, S, E865 |
| Ninomiya, S, E1244, P202 |
| Ninomiya, S, PB1708 |
| Ninomiya, S, PB2158 |
| Nollet, F, E1056 |
| Nöllke, P, P350 |
| Nomdedeu, B, E1181 |
| Nomdedeu, J, S790, P202 |
| Nomdedeu, J, P759, P765, E831, E1026, PB1668, PB1782 |
| Nomura, S, P6711 |
| Nonino, A, PB2025 |
| Nooka, A, S459, P335 |
| Noonan, K, S779 |
| Nord, C, E1439 |
| Norell, H, E889 |
| Norfo, R, S421 |
| Nørgaard, J, P193, E932 |
| Nørgaard, M, P193, E932, E1435 |
| Noria, A, E1273 |
| Noris, P, S432, S433, P365 |
| Noroozi-Aghideh, A, P309 |
| Norvaly, S, P269 |
| Norris, B, P194 |
| Norris, M, P154 |
| Norton, A, P668 |
| Norvila, R, E1311 |
| Nosari, A, P404 |
| Nta, T, P404 |
| Notarangelo, L, P717, E1420 |
| Notario McDonell, C, E983 |
| Nóthen, M, P295 |
| Novák, J, E936 |
| Novak, R, S147 |
| Novak, S, S150 |
| Novak, V, PB2238 |
| Nováková, M, E1376 |
| Novakovič, S, PB1706 |
| Novella, E, S485 |
| Novell, E, E1481 |
| Novelli, S, S963, PB1887, PB2159 |
| Novkovic, A, PB1871 |
| Novo, A, PB1616 |
| Novo, S, E1577 |
| Novoa, V, E1182 |
| Novosad, O, E1382, E1127, PB2069 |
| Novszewska-Kania, M, E886 |
| Nuzzoli, C, E1215, E1270, PB1983 |
| Ntanasis-Stathopoulos, I, E1249 |
| Ntoufta, S, P244 |
| Nucifora, E, E1182 |
| Nudelmam, M, P691 |
| Nudelmam, O, P548 |
| Numata, A, PB1971 |
| Nunez, A, P654 |
| Nuñez Cortés, A, E1500, PB2219 |
| Nunn, M, P235 |
| Nur, E, S455 |
| Nwa, D, P2206, PB2213, PB2227 |
| Nwog, B, P1909 |
| Nyold, C, E1367 |
| O'Brien, M, P521 |
| O'Brien, S, S423, P591 |
| O'Brien, S, S463, S769, S772, P170, P209, P240, P253, P518, P519, E991, E1016 |
| O'Byrne, S, P270 |
| O'Connell, A, P204 |
| O'Connor, D, P668 |
| O'Connor, P, E1147 |
| O'Dwyer, M, P203, P547 |
| O'Keeffe, D, PB2014 |
| O'Meara, M, P201 |
| O'Meara, S, E1201 |
| O'Neill, B, PB1898 |
| O'Reilly, R, P745 |
| O'Rourke, L, P334 |
| O'Sullivan, J, E1550 |
| O'Toole, R, P2106 |
| O'hara, C, P663 |
| Oakes, R, P225 |
| Obara, M, E859 |
| Obara, N, E1519 |
| Obeka, N, PB2213, PB2227 |
| Oberbeck, S, E1376 |
Ribera, J, S790, P202
Ribera, J, E831, PB1616
Ribera, J, E1086
Ribera, J-M, E831, E1394, E1396, PB1616, PB1991
Ribolla, R, S102
Ribrag, V, S105, S467, P344, E1018
Ricardo, A, S500
Ricchi, P, S129, E1018, E1570, E1571, E1575
Ricci, F, E1048
Ricciardi, M, PB1696
Ricco, A, P598, E1560
Rice, L, E950, E1146
Richard, S, E1470
Richard Espiga, C, S798
Richardson, D, S817
Richebourg, S, E1361
Richez, V, PB1987
Richter, J, S426, P593, E1057
Richter, J, E1276, E1288
Ricker, J, P634
Rico, A, P751
Ricordeau, I, P712
Rider, A, E1291, PB1687, PB1699
Ridley, A, PB214
Ridgway, B, E1470
Riel, S, E1394
Riestra, J, E1396
Riesz, A, E1508
Rivadeneira, L, E1508
Rivas, M, E1508
Rivas-Delgado, A, E1032, E1137
Rivas Vera, M, PB1738, PB1866
Rivas-Vera, S, E1473
Rivier, S, PB1696
Rivoli, G, E1299
Rivotto, B, S779
Riwes, M, P753
Rizea, O, PB1642
Rizki, S, PB2029
Rizo, A, E1322
Rizvi, S, E1248
Rizzi, D, P1919, P556
Rizzo, D, E1010
Rizzo, J, E1451, E1456
Rivere, I, S143, S479
Rivière, J, E1008
Rivoltella, V, S779
Rivolta, B, E1278
Rivoira, J, E1451, E1456
Rivera, V, P603
Rivera-Fong, L, E1473
Riveros Rosas, A, E1473
Rives, S, S476, P168, P517
Rivière, J, E1275
Rivoli, G, E1299
Rivotto, B, S779
Rizzi, D, E1010
Rizzo, J, P745
Rizzo, M, E1281
Rizzo, M, E1379
Rizzuto, V, S467, P344, E1018
Rokab, P, E1262
Rokab, T, P235, P248
Robert, D, P62201
Roberts, C, S791
Robertson, J, E866
Roberts-Rapp, L, P682
Robillard, N, PB1615
Robinson, K, PB1613
Robinson, M, PB2146
Robinson, S, S502, E874
Robledo, C, P318, P510, P655, E1014, E1169
Robles, A, P554
Robles, M, PB1629
Robles García, R, E1473
Rozzi, S, S476, S479, E1169
Rombach, S, S486
Rocchi, S, E1171, E1283, PB1974
Roca, R, E1171
Roca, S, E1075
Rocamora, G, P290
Roccario, A, E1354
Rodar, B, E1417, E1555
Rodin, S, P610
Rodi, S, P564
Rodriguez-Alvarez, A, P564, E1113
Rodríguez, A, E1349
Rodríguez, B, E1592
Rodriguez, C, P751
Rodríguez, J, P212
Rodríguez, M, E1281
Rodríguez, N, E1349
Rodríguez, R, PB1661
Rodríguez-Caballero, A, E1019
Rodríguez-García, G, E1113
Rodríguez-Fernández, A, E1238
Rodríguez-Hernández, A, E1239
Rodríguez-Lobato, L, E1259
Rodríguez-Macías, G, P554, E1113, PB1653
Rodríguez-Otero, P, S409, S783, P888
Roter, C, E1417, E1555
Roter, S, P610
Ruiz Arguelles, A, E1500
Ruiz Arguelles, M, E1500
Ruiz Argüelles, G, E1500, PB2219
Ruiz-Cabello, F, P696
Ruiz Delgado, G, E1500, PB2219
Ruiz Delgado, R, E1500
Ruiz Garcia, E, PB1738
Ruiz-Heredia, Y, P679, E891, E899, PB1912
Ruiz-Lloret, A, P168
Ruiz Reyes, G, E1500
Ruiz-Xiville, N, S461, PB1616
Rukavitcyn, A, PB1865
Rukavitcyn, O, PB1865
Ruland, J, S818
Rule, S, E1377
Rumi, E, P351, P357, E1337
Rummell, C, E1393
Rupa-Matysek, J, E1079
Ruparelia, M, PB1836
Rupoli, S, P636, E1136
Rupp, J, E992
Rusinov, M, E853, E860, PB1615
Rusinov, M, E836, E1227
Ruskin, A, PB1829
Russell, J, P276, PB2057
Russell, N, S114, S407, S781
Russell, S, P331, P673, E1240
Russiñol, N, P117, P325
Russo, A, PB2038, PB2039, PB2056
Russo, D, P607, P705, E1325
Russo, F, P337, P684, E1243
Russo, G, P715, P717, E1164, E1491
Russo, L, P760, P766
Russo, M, E1263
Russo, R, S811
Russo, S, P604
Russo Rossi, A, S485, P599, P604, E1560, PB1818
Rutten, A, E874
Ryabchikova, N, PB1803
Ryabukhina, Y, P220, E1133
Ryan, J, P508
Ryan, K, S481
Ryan, R, S808, P210, P556, P740, E922
Ryan, R, E994
Rybakova, L, P308, E1568
Rybicka-Ramos, M, P741
Rybka, E, P1553, PB2032
Rydzanicz, M, PB1802
Rydzek, J, S816
Ryland, G, E1202
Rymikiewicz, G, P162
Ryoo, NH, E871
Ryu, D-B, P757
Ryzhak, O, E1127
Ryzhikova, N, E1403, E1409
Ryznerova, P, PB1766
S
Saad, H, PB1767
Saad, S, E1494, PB1674
Saadeh, C, PB2177
Saadoun, H, P649
Saavedra, S, PB1782
Sabañez, E, P20286
Sabater-Leal, M, P759, P765
Sabatini, F, P658
Sabatino, M, P523
Sabinou, F, PB1987
Saccà, V, P644
Saccardi, R, P8193
Saccetti, E, E1020
Sacchi, M, S422
Sacchi, N, S796
Saccone, A, E1354
Saccomi, A, E1379
Sachdeva, M, E987
Sadhak, K, P292
Sadeghi, B, P379
Sadelain, M, S143, S479
Sadil, S, P258
Sadadjan, R, P702
Sadoudi, S, P617
Sadri, S, E1159, PB1701, PB1746, PB1759
Sadullah, S, S502, P276, PB2057
Sadov, N, P307
Saeed, B, P179, P206
Saeed, H, P279
Saes, I, P657
Sáez Salinas, A, E1327
Saez-Perdomo, M, PB2174, PB2179
Saft, L, P486
Safta, G, PB1642
Safuanova, G, PB1803
Sagi, R, P689
Sagig, G, S485, P599, P601, P605, E1060, PB1818
Sagou, K, E1516
Sagüés, M, PB2111
Sah, C, P276
Saha, V, E1485
Sahakyan, L, PB1643
Sahir, D, S474
Sahir, F, E1525, PB1968
Said, Q, P285
Said, Z, PB2236
Saif, M, S817
Saikia, T, S422, E1062
Sail, K, P728, E1466
Saillard, C, P534
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, PB2158
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
Sal, A, PB2087
Sali, A, E955
Sala, E, P544, PB1878
Sala, E, PB2236
Salam, H, E1514, PB2072
Salamero, O, S790, P202, P554, PB1668
Salamounbat, 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
Salcioglu, Z, E1418, PB1640, PB1649
Saleh, M, P723, E1437
Salehzadeh, S, E1048
Salek, C, E841
Wicherska-Pawłowska, K, E1553
Wichmann, C, S427
Wichmann, M, S494
Wieland-Greguare-Sander, A, E1455
Wiemels, J, P300
Wierda, W, S463, S771, S772, P241, P518, P523, P564, P728, E991, E1016, E1466
Wierzbowska, A, E1022
Wierzbowska, A, E920
Wiesenfarth, M, P350
Wiesneth, M, E1084
Wiestner, A, P588, E1369
Wiezorek, J, S466, P522, P523, E840
Wiggers, C, P187, E901
Wiggill, T, E1405
Wigley, C, P368
Wihlborg, A-K, E826
Wijatyk, A, PB1631
Wildenberger, K, P261
Wilhelm, A, P526
Wilke, T, E842
Wilkes, E, P532
Will, B, S135
Will, R, P663
Willard-Gallo, K, E994
Wille, K, E1335
Willemze, R, E1362
Willenbacher, E, P707
Willenbacher, W, S782, PB1988
Williams, C, S407, S781
Williams, S, E1509
Wimperis, J, P276
Windyga, J, S435, P235
Wingett, S, S117
Winiarska, M, S125, E1359, E1385
Winograd, B, P332
Winstone, D, E1534
Winter, M, P392
Witt, O, P663
Witte, B, S501
Witte, T, P350
Wittner, M, E1102
Witzens-Harig, M, E839
Wolach, O, E949
Wolf, A, S419, E879
Wolf, P, E1110
Wolter, P, P565, P637
Wolz, O, E1356
Won, J-H, E894, E1608
Won, Y-W, P259, E939, E1054
Wong, G, P646
Wong, K, E1354
Wong, N, E847
Wong, R, P723, E1437, PB1679
Wong, S, E1146
Wong, S, E1140
Wong, W, E1073
Woo, A, S147
Wood, B, S793
Wood, E, P674, PB1988
Wood, M, S147
Wood, P, S476, S477, P517
Woodman, R, PB1815
Woods, G, P620
Woolfrey, A, S146, P381
Woolworth, J, E1073
Wormann, B, E885
Woyach, J, E1400
Wozniak, J, E886
Wozniacz-Karczmzar, I, PB1744
Wray, K, P228, E1424
Wrench, B, S802, P160, P164
Wright, L, P295
Wrigley, B, S469
Wrobler, T, E1553, PB1695, PB1889, PB2032
Wrodnigg, T, E1395
Wu, C, P236
Wu, C-J, S500
Wu, D, P390, E928, E931, E956, E1152, E1189, E1507, E1520, E1521, E1531, PB1635, PB1977, PB2041
Wu, D-P, E1144
Wu, H, PB1719
Wu, J, S138
Wu, J, P704
Wu, J, P753
Wu, K, S101, P334, P335, P676
Wu, M-C, PB1734, PB1737
Wu, N, E1073
Wu, N, E1567
Wu, X, P390, E1520
Wu, X, PB1671
Wu, X, PB1752, PB1753
Wu, Y, P719
Wu, Y, P224
Wu, Y, PB2093
Wuchtler, P, P172
Wulf, G, P226, E668, E670
Wust, T, S132
Wyn, C, P568
Wylie, B, E1350
Wynn, R, S817, P668, PB2168
Wypasek, E, S445
X
Xekalou, A, PB1961
Xia, Z, E1861, E1389
Xiang, B, P224
Xiangmeng, W, PB1654
Xiao, L, P209
Xiao, V, E1073
Xiao, X, E1521
Xiao, Y, P285
Xiao, Z, P356, E1330
Xicoy, B, P609, E1181
Xie, J, PB2135
Xie, L, P224
Xie, Y, E1520
Xie, Z, S108, P197, E921
Xie, X, E1419, P1916
Xing, Y, E950
Xingzhi, S, S489, P207
Xiong, B, S490
Xisto Souto, E, PB1856
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Late Breaking Oral Session

**LB2601**

**CRYPTIC INSERTIONS OF IMMUNOGLOBULIN LIGHT CHAIN ENHANCER REGIONS ACTIVATE CCND2 AND CCND3 IN CYCLIN D1-NEGATIVE MANTLE CELL LYMPHOMAS**

D. Martin-Garcia1, A. Navarro1, G. Clot1, I. Ribera-Cortada1, B. González-Ferrari1, J. Gutiérrez-Abri2, R. Valdés-Mas2, R. Woroniecka3, G. Rymkiewicz3, D. Torrents4, S. Beltran5, B. González-Farré1, E. Pikarsky1, Y. Ben-Neriah1,*, F. Mercurio3, M. Oren4, E. Pikarsky1, Y. Ben-Neriah1, 1Immunology and Cancer Research, Hebrew University of Jerusalem, Jerusalem, Israel, 2Chemistry, WuXi AppTec, Shanghai, China, 3BioTheryX Inc, San Diego, United States, 4Molecular Cell Biology, Weizmann Institute, Rehovot, Israel

**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 CKIα robustly activates p53 (doi:10.1038/nature09673). However, with no selective CKIα inhibitors for in vivo use, the therapeutic value of CKIα inhibition in hematological malignancies cannot be validated.

**Aims:** To develop small molecule CKIα inhibitors and assess their effect in mouse models of human leukemia.

**Methods:** CKIα inhibitors were identified via cell-based screening based on p53 activation. We focused on a small class of pyrazolo-pyrimidine scaffolds, which through extensive medicinal chemistry yielded derivatives with high affinity binding, validated by crystallography studies, potent CKIα 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).

**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 leukemic 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 HPSCs was achieved in these mice up to 5 months' observation, 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 HPSCs was achieved in these mice.

**Summary/Conclusions:** We developed a new class of small molecule inhibitors that co-target CKIα and P-TEFβ. 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**

**NOVEL SMALL MOLECULE INHIBITORS CO-TARGETING CK1A AND P-TEF-B DISRUPT SUPER-EnhANCERS AND ERADICATE ACUTE MYELOID LEUKEMIA IN A MOUSE MODEL**

W. Minzeli, 1, A. Alkalay1, A. Fink1, V. Venkatchalamar1, E. Hung1, D. Li2, J. Vaccara2, F. Mercuno3, M. Oren4, E. Pikarsky1, Y. Ben-Neriah1, 1Immunology and Cancer Research, Hebrew University of Jerusalem, Jerusalem, Israel, 2Chemistry, WuXi AppTec, Shanghai, China, 3BioTheryX Inc, San Diego, United States, 4Molecular Cell Biology, Weizmann Institute, Rehovot, Israel

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**Summary/Conclusions:** We developed a new class of small molecule inhibitors that co-target CKIα and P-TEFβ. 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.
Mental hypoxia and high-risk 1q gain in multiple myeloma

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. 1q 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.

Methods: Whole-exome sequencing (WES) or targeted next generation sequencing (NGS) were employed for extensive phenotyping of WT and CD69-/- NK cells and RNAseq analyses were used to elucidate the molecular mechanisms implicated. Mouse strains deficient in T cells, B cells and NK cells were used to establish the NK cells as the population responsible for the observed phenotype. Mass cytometry was performed on CD69-/-mice were highly resistant to aGvHD and significantly more efficient at eliminating hyper-reactive allogeneic T cells and confer resistance to aGvHD. This data could pave the way for novel therapeutic strategies to optimize allogeneic HSCT.

Summary/Conclusions: Together, these findings argue that HIF-1β represents a potential marker for risk stratification and prognosis 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.

LB6206

ANTI-CD69 MAB TREATMENT INCREASES THE CAPACITY OF NK CELLS TO ELIMINATE HYPER-REACTIVE ALLOGENIC T CELLS AND PREVENTS ACUTE GRAFT VERSUS HOST DISEASE

Background: CD69 is a C-type lectin expressed on the surface of certain immune cell progenitors as well as activated mature leukocytes. CD69-/- mice 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 allogenic T cells and whether this would lead to successful aGVHD prevention.

Methods: We took advantage of a fully allogenic aGVHD mouse model in which wild type (WT) or CD69-/- BALB/c mice were lethally irradiated and reconstituted with C57/Bl6 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 population responsible for aGvHD prevention. 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.

Results: CD69-/- mice were highly resistant to aGvHD and significantly more efficient at eliminating hyper-reactive allogenic 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 upregulate 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 allogenic T cells and confer resistance to aGvHD. This data could pave the way for novel therapeutic strategies to optimize allogeneic HSCT.

LB6204

GLOBAL PIVOTAL PHASE 2 TRIAL OF THE CD19-TARGETED THERAPY CTL019 IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA – AN INTERIM ANALYSIS

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: Methods: To determine expression of factor-1β (HIF-1β), a gene located in the 1q21 region, in 1q MM and hypoxic MM cells, Western blot and qPCR analyses were performed to determine expression of HIF-1β and other 1q21 genes in 1q MM and hypoxic 1q MM cells, 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.

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=0.338), among which 24.3, 43.3, and 43.8 months for 1q copy number ≥3, ≥4, and ≥5 (P=0.030), respectively; whereas Btz-based therapy displayed a marked increase in response rate ≥VGPR, 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 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 reproduced in vivo. Expression of HIF-1β and other 1q21 genes in 1q MM and hypoxic MM cells, 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.

Summary/Conclusions: Together, these findings argue that HIF-1β represents a potential marker for risk stratification and prognosis 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: 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 confirms the high response rates and durable remissions observed in the previous single-center experience in a cohort of highly refractory pts. Efficacy was observed in 57% of infused pts (17% grade 3; 9% grade 4); no CRS-associated deaths were reported.

Methods: Industry-manufactured CAR T-cell products were provided to 26 centers on 4 continents using a global supply chain. Pts had received ≥2 lines of chemotherapy and had disease progression after or were ineligible for autologous stem cell transplant (autoSCT). Autologous T cells were transduced with a lentiviral vector encoding an anti-CD19 CAR, expanded, cryopreserved, shipped, and infused at study sites. The primary endpoint (centrally reviewed CR by an independent review committee) was best overall response rate (ORR: CR + partial response [PR]).

Results: 141 pts were treated. Following restaging, bridging therapy, and lymphodepleting chemotherapy (fludarabine 25 mg/m²/cycle and cyclophosphamide 250 mg/m²/day × 3 days or bendamustine 90 mg/m²/day × 2 days), 85 pts received a single dose of CTL019 transduced cells (median, 3.1 × 10⁸ [range, 0.1 - 1.0] × 10⁸ cells). Median time from infusion to data cutoff (20 December 2016) was 7 mo (range, 0.1 - 36 mo). CR and PR rates at 3 mo were 37% and 8%, respectively. All pts in CR and 11% of pts in PR at data cutoff had disease 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 responses observed in the previous single-center experience in a cohort of highly refractory pts. Industry-manufactured CAR T-cell products were feasible. CRS and other AEs were effectively managed by appropriately trained investigators.

Background: BM adipocyte content may help leukemia escaping drug treatment. BM adipose tissue is a dynamic microenvironment with the BM mesenchymal compartment generating pluripotent cells that are able to generate BM adipocytes (BM-ADPs). BM-ADPs are directed to BM niches which shape the leukemia burden by altering host metabolism and by recruiting BM progenitors. BM-ADPs can then be mobilized to engraft distant BM sites where they contribute to T-ALL evolution. The underlying molecular mechanisms are still not fully understood.

Methods: We used grafts of human and mouse T-ALL in immune-deficient and humanized mice to follow T-ALL evolution and to study the role of BM-ADPs in T-ALL evolution. The BM niche was engrafted with BM adipocytes obtained from the BM of T-ALL mice. BM-ADPs were isolated based on their adipocyte morphology and their ability to express CD34 and CD45f and display dynamic endothelial to hematopoietic transcription programs. In addition, reprogrammed fibroblasts repopulate immunodeficient NSG mice and generate hematopoietic progeny of multiple lineages, including T-cells and myeloid cells. Mechanistically, GATA2 display dominant and independent targeting activity during the early phases of reprogramming while GF11B interact and co-occupy a cohort of target sites engaging sites preferentially with AP-1 motifs, including the RUNX1 locus. This cooperative binding is reflected by the engagement of open enhancers and promoter marks by H3K4me3, H3K4me1 and H3K27ac in the fibroblast genome as well as the silencing of fibroblast genes while activating the hematopoetic program.

Summary/Conclusions: Together, these findings uncover a collaborative TF interaction that specify a human hematopoietic program and EHT. These findings shed light on the processes controlling human HSC specification and provide means to generate human reprogrammed HSCLs at high efficiency for transplantation.

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/TLX3 overexpression or SILT1 deletion are known to induce survival, proliferation and differentiation block in T-ALL cells. Interactions between leukemic cells and their microenvironment also contribute to T-ALL pathogenesis. Cell-cell contacts - Delta-Like/Jagged-Notch1, integrin LFA1/ICAM1 - and secreted factors - such as interleukin 7 and 15, transforming growth factor β, are key players in the T-ALL development. In the course of the disease, T-ALL cells settle in various environments such as thymus, blood, bone marrow (BM), pleura or lymph nodes, which differ in terms of cell content, extracellular matrix and secreted factors. To which extent these distinct niches imprint niche-specific features on T-ALL cells is not well understood.

Aims: Compare the growth of leukemic cells from human and mouse T-ALL in various BM sites. Uncover novel mechanisms of chemoresistance, in relation with the BM microenvironment.

Methods: We used grafts of human and mouse T-ALL in immune-deficient and normal mice, respectively. We explored the behavior of leukemic cells ex-vivo and in vivo after they had engrafted different BM sites of the mouse body (femurs, Thorax and Tail vertebrae). We tested their respective chemoresistance to conventional drugs (dexamethasone, vincristine, cytarabine).

Results: We observed that mouse and human T-ALL develop slowly in tail vertebrae BM compared to thorax vertebrae and femur BM. T-ALL recovered from tail BM display lower cell surface marker expression and decreased metabolism and cell cycle progression, demonstrating a dormancy phenotype. Functionally, tail-derived T-ALL exhibit a deficient short-term ex vivo growth and a delayed in vivo propagation. These features are non-cell autonomous as T-ALL from tail and thorax share identical genomic abnormalities and functional disparities disappear in vivo and in prolonged in vitro assays. Importantly, tail-derived T-ALL display a more intrinsic resistance to drugs such as vincristine, cytarabine and corticosteroids, but not to dexamethasone. T-ALL recovered from gonadal adipose tissues or from co-cultures with adipocytes share metabolic, cell cycle and phenotypic or chemoresistance features with Tail-derived T-ALL.

Summary/Conclusions: These results demonstrate that BM sites differentially impact T-ALL propagation. Tail-derived T-ALL is derived from adipocytes associated with quiescence and decreased response to cell cycle dependent chemotherapy indicating that adipocyte-rich aged BM or pathologies enhancing BM adipocyte content may help leukemia escaping drug treatment.
Index of authors

A
Alkalay I LB2600
Anak Ö LB2604
Andreadis C LB2604
Arcangeli M-L LB2606
Awasthi R LB2604

B
Bachanova V LB2604
Balas A LB2603
Ballerini P LB2606
Baruchel A LB2606
Bea 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
Clot 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 J 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-Otín C LB2601

M
Ma’ayan A LB2605

Magenu JM LB2604
Martin P LB2603
Martín-Garcia D LB2601
Matutes E LB2601
Maziarz RT LB2604
McQuirk 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-Martinez L LB2601

R
Relano M LB2603
Ribera-Cortada I LB2601
Rosenwald A LB2601
Rymkiewicz G LB2601

S
Salaverria 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
Tsiligiri 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