The impact of the introduction of bortezomib on dialysis independence in multiple myeloma patients with renal failure

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PRESCRIBING INFORMATION – Iclusig®

Indications:

Iclusig® is the 3rd generation TKI with the potential to deliver FAST, DEEP and DURABLE response in patients of concern with CML.1

At 4 years, 23% of chronic-phase CML patients achieved a deep molecular response (MR4.5) with Iclusig®.1

Consider dose reduction to 15 mg for CP-CML patients who have the T315I mutation.

Most severe events occurred in first 3 months; overall, events occurred more frequently in AP-CML, BP-CML or Ph+ ALL than CP-CML. Caution with use of anti-clotting agents.

Most common serious ADRs (see SmPC for details of recommended monitoring and management. Myelosuppression: Perform Full Blood Count every 2 weeks until normalised. Monitor for neutropenic fever.

HAEMATOLOGIC TOXICITY

Myelosuppression: Severe neutropenia (ANC <0.5 x 10⁹/l) has occurred in 4% of patients. Normalise ANC before starting treatment. Consider dose reduction to 15 mg in patients who have the T315I mutation.

Pancreatitis and serum lipase: check serum lipase fortnightly for 2 months and then periodically.

Effects on ability to drive and use machines

Lethargy, dizziness, fatigue, headache, dizziness, hypotension, diabetes, or hyperlipidaemia.

Drug Interactions: see SmPC for details.

Pregnancy and breastfeeding:

Advise patients not to become pregnant or father a child during treatment; use effective contraception. Studies in animals have shown reproductive toxicity. Breastfeeding should be discontinued.

Hepatitis B reactivation: test for HBV before treatment.

References:


Contraindications:

- Severe haematological and non haematological toxicities; consult the SmPC for full details of recommended monitoring and management.
- Intestinal Tumours, adrenocortical carcinoma or small cell lung carcinoma.
- Pregnancy and breastfeeding

CAUTION

Consider other treatment options in patients with prior myocardial infarction.
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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
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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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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!
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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
New advances in plasma cell disorders and implications for therapy

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

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

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

Results: Based on the RNA-Seq and exome results, cytogenetic risk status in the CASTOR and POLLUX studies was defined as high risk with having 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;14), and del17p in both studies (Table 1).

<table>
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<th>Concordance rate between FISH and NGS</th>
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<th>CASTOR</th>
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<tr>
<td>t(4;14)</td>
<td>96%</td>
<td>95%</td>
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<tr>
<td>t(14;14)</td>
<td>96%</td>
<td>97%</td>
</tr>
<tr>
<td>del17p</td>
<td>96%</td>
<td>99%</td>
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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;14), and del17p and showed good concordance with FISH. As FISH was performed locally with different probes and pathologists, the high degree of concordance between FISH and NGS is notable and supports the use of NGS for determining cytogenetic risk in patients with RRMM. The utility of NGS in these clinical studies extends far beyond the detection of cytogenetic abnormalities and additional analysis are planned to interrogate these datasets in the identification of novel biomarkers.
significantly improved progression-free survival (PFS) and achieved higher overall response rates (ORRs) compared with the respective standard-of-care regimen alone (Dimopoulos MA et al., N Engl J Med 2016;375(14):1319-1331; Palumbo A et al., N Engl J Med 2016;375(8):754-766.). Due to its novel mechanisms of action, addition of D to standard-of-care regimens may benefit RRMM patients who have poor prognoses resulting from high-risk cytogenetic abnormalities.

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

**Methods:** Bone marrow aspirates were collected at screening visits from 311/569 patients from POLLUX and from 353/498 patients from CASTOR, and cytogenetic abnormalities were detected via next-generation sequencing (NGS). Patients were considered to be of high cytogenetic risk 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=0.014). Responses to DRd vs Rd included CR or better in 33% vs 6% of these patients, and very good partial responses (VGPR) or better in 63% vs 31%. In standard-risk patients, DRd vs Rd also resulted in significant improvements in median PFS (Figure 1) as well as ORR (95% vs 82%; P=0.0020). Responses to DRd vs Rd included CR or better in 52% vs 24% of these patients, and VGPR or better in 84% vs 51%. At 10^{-5} sensitivity threshold, MRD-negative rates for DRd vs Rd were 18% vs 0% (P=0.0027) among high-risk patients and 30% vs 10% (P=0.0001) for standard-risk patients. For CASTOR, the median follow-up was 13.0 months. Treating both high- and standard-risk patients with DVd vs Vd significantly prolonged median PFS (bottom panel Figure 1) and increased ORR (high risk: 82% vs 62%; P=0.039; standard risk: 85% vs 64%; P=0.0003). Responses to DVd vs Vd among high-risk patients included CR or better in 30% vs 9% of patients and VGPR or better in 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


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**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) or 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 or more 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 MRD enrolment, 76% (239/316) patients were MRD-negative: 64% (153/239) in the HDM vs 36% (86/239) in the VMP groups. The 3-year PFS was 50% in MRD-positive vs 77% in MRD-negative patients (HR 2.87, 95% CI: 1.75 - 4.72, p<0.001). Subgroup analyses were carried out to assess the risk factors for MRD-positivity according to baseline characteristics and therapies: high risk cytogenetic abnormalities were the most important risk factors (HR 9.87, 95% CI: 4.3 – 22.63; interaction p=0.001). Finally, 48% of MRD positive patients at pre-maintenance who had a second MRD evaluation after at least 1 year of lenalidomide became MRD-negative.

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

S103

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

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

**Aims:** This phase I, open-label trial was conducted to assess the efficacy and
safety profile of LCAR-B38M anti-BCMA CAR T cells in patients with relapsed/refractory multiple myeloma. **Methods:** All patients underwent leukapheresis to obtain peripheral blood mononuclear cells and their T cells were engineered to express anti-BCMA CAR. Three doses of 300 mg/m² cyclophosphamide were administered on day -5, -4, and -3 (before recruitment, patients took the same chemotherapy to identify they were refractory to cyclophosphamide monotherapy) and engineered T cells were rein infused on day 0, 2, and 6. This trial was divided into the dose escalation stage and expansion cohort. Toxicity and responses were assessed according to the Common Terminology Criteria for Adverse Events (version 4.0) and International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma, respectively. **Results:** As of the February 20th, 2017 data cut-off, 22 patients had been enrolled, two of whom were diagnosed as plasma cell leukemia. The male: female ratio was 11:11 and median age was 53.5 years. Chromosomal abnormalities were detectable by FISH in eight patients, two of whom involved in the deficiency of p53. Eleven patients were triple refractory (chemotherapy, proteasome inhibitors, and immunomodulatory drugs), 11 resisted to double prior treatments (chemotherapy and proteasome inhibitors/ immunomodulatory drugs), and four relapsed after autologous hematopoietic stem cell transplant. The median number of infused CAR T cells was 4.0×10^6 (range, 1.5×10^5 to 7.0×10^6) per kg. The median follow-up was 131.5 months (range, 29-327) days. 100% of patients achieved an objective response. The first six patients achieved complete responses with flow MRD-negative; 14 patients achieved very good partial responses; one patient, with renal failure, achieved partial response; all these 22 patients had kept their best response at the end of follow-up. The pictures we enclosed were the subcutaneous nodules in one patient with extramedullary plasmacytoma. We found that the nodules were obviously decreased after the infusion and disappeared finally. Another one achieved transient partial response, which last for 12 days. He then took the secondary infusion but failed since the post-operation large-dose administration of corticosteroid for spinal meningoma. He terminally died of the progression of myeloma. The most common toxicity attributable to CAR T cells was cytokine release syndrome (CRS). Toxicities were minimal except for two grade 3 CRS and one grade 4 CRS. All CRSs were controllable with nonsteroidal anti-inflammatory drugs (NSAIDs) or tocilizumab and no dose-limiting toxicities or treatment-related deaths were observed (Figure 1).

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

**S104**

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

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

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

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

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

**Results:** In the overall population (N=69), the median age was 61 years (61% male). Median (range) time since diagnosis was 2.9 (0.4-16.0) years, and 45% of patients underwent ≥3 previous PCD regimens. Median time to first best response was 1.8 (cardiac), 3.7 (renal), and 1.0 (PN) months. Best response rate indicating organ response was observed in 53% of cardiac-evaluable patients (n=19/36) and 64% of renal-evaluable patients (n=23/36). PN responses were observed in 82% (n=9/11) of PN-evaluable patients. Time from patients’ best HR to previous PCD treatment was not related to the attainment of NEO001 organ response (responder/ stable: 35.6/36.6 months [cardiac] and 30.6/32.5 months [renal]; P>0.05). Depth of patients’ best HR also was not related to the attainment of NEO001 organ response (percentage of patients with organ response in CR/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 meningoma: stem cell transplantation, 55.6/61.1% [cardiac/renal]; bortezomib-based therapy, 52.0/68.8%; or other chemotherapy, 50.0/57.1%; P>0.05). Exploratory analyses showed no association between the time to response or percentage of responders and the number of previous PCD treatments.

**Summary/Conclusions:** NEO001 specifically targets disease-causing, misfolded LC aggregates in AL amyloidosis. Organ responses in patients treated with monthly NEO001 infusions were achieved rapidly and independently of time since previous chemotherapy, depth of hematologic response, or predominant type of PCD treatment.
**Aggressive Non-Hodgkin lymphoma - 1° line**

**S105**

**RITUXIMAB MAINTENANCE AFTER AUTOLOGOUS TEM CELL TRANSPLANTATION PROLONGS SURVIVAL IN YOUNGER PATIENTS WITH MANTLE CELL LYMPHOMA: FINAL RESULTS OF THE LYMA TRIAL OF THE LYSA/GOELAMS GROUP**

**Methods:**
In patients with DLBCL.

**Aims:**
We evaluated the safety and efficacy of pola-R-CHP as first-line treatment in 1L patients with MCL, an acceptable profile and potential improvement in response rates at the end of treatment.

**Results:**
4 courses of immunochemoetherapy induction (R-DHAP); Rituximab, cyclophosphamide, doxorubicin, prednisone for patients with previously untreated diffuse large B-cell lymphoma. The primary end point was event-free survival (EFS) (progression, relapse, death, severe infection during RM) after ASCT.

**Summary/Conclusions:**
The LyMa trial demonstrates for the first time that RM after ASCT prolongs EFS, PFS and OS. Thus, 4 courses of R-DHAP plus ASCT (without TBI) followed by RM maintenance (one infusion every 2 month for 3 years) is a new standard of care for young MCL patients.

**References**

**S106**

**POLA-R-CHP: POLATUZUMAB VEDOTIN COMBINED WITH RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN, PREDNISOLONE FOR PATIENTS WITH PREVIOUSLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA**

**Methods:**
In patients with DLBCL.

**Aims:**
- To evaluate the safety and efficacy of pola-R-CHP as first-line treatment in patients with DLBCL.
- To evaluate the safety and efficacy of pola-R-CHP as first-line treatment in patients with DLBCL.

**Results:**
- Grade ³3 adverse events (58.3% SC; 54.3% IV) and administration-related reactions (CR) were also similar at 24 months’ follow-up (non-significant differences; Table 1).
- RASQ scores
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>Rizumab SC plus COP vs</th>
<th>Rizumab IV plus COP vs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR/PR</td>
<td>N (%); 95% CI</td>
<td>N (%); 95% CI</td>
</tr>
<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>50.6 (42.3–53.9)</td>
<td>42.4 (34.8–50.0)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>31.8 (25.7–38.8)</td>
<td>28.6 (21.9–35.1)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>31.1 (25.0–38.1)</td>
<td>31.1 (25.0–38.1)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>82.1 (77.8–86.6)</td>
<td>78.4 (73.5–83.3)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>61.0 (53.0–69.1)</td>
<td>61.0 (53.0–69.1)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>67.4 (61.5–73.3)</td>
<td>67.4 (61.5–73.3)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>67.4 (61.5–73.3)</td>
<td>67.4 (61.5–73.3)</td>
<td></td>
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<tr>
<td>N</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>67.4 (61.5–73.3)</td>
<td>67.4 (61.5–73.3)</td>
<td></td>
</tr>
</tbody>
</table>

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

**S108**

**ANALYSIS AND CHARACTERIZATION OF HEMATOLOGIC CANCERS USING A COMPREHENSIVE NGS PANEL COMPRISING OF DNA AND RNA TARGETS TREATING 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). 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 using the NIST human reference sample NA12878 and a comprehensive panel of hematologic cancer derived cell lines with known pathogenic variants at various allelic frequencies.

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

**Summary/Conclusions:** MyHEME is an extensive panel for sensitively and specifically identifying SNV, indel and SV mutations in 704 target genes. This panel can comprehensively characterize mutations in multiple diverse hematologic cancer samples, including Non-Hodgkin Lymphoma (NHL). We cross-confirmed when observed in both the DNA and RNA targets. Importantly, novel gene fusions, which are generally difficult to detect and validate, were cross-confirmed when observed in both the DNA and RNA targets. For example, we identified a novel t(9;22) translocation causing a NUP214-XXR3 gene fusion using both the DNA and RNA targets. Additionally, while RNA data provides the fused exons of the transcripts, DNA data gives the precise genomic breakpoint coordinate.

**S109**

**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 were 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% in 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 <6 years (median 58, range 37-65). Fifty-three percent were either MIP intermediate- or high-risk, 17% had blastoid morphology and 42% had Ki67>20%, 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 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 deletions of TP53 in 29 patients (13%) showed that del-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>20%. Del-TP53 was associated with Ki67>20%, 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 abervations. 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>20%, del-CDKN2A and del-CDKN2A) only mutations of TP53 remained a significant predictor of outcome.

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

S110

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


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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. Giltertinib, a highly selective FLT3/AXL inhibitor, has displayed antileukemic activity in FLT3 mutation-positive (FLT3mut+) relapsed/refractory (R/R) AML in the CHRYSSALIS Phase 1/2 study (NCT02014558), specifically at doses ≥80 mg/d. Aims: To assess molecular response to giltertinib in a CHRYSSALIS subpopulation.

Methods: This exploratory analysis evaluated molecular response in patients aged ≥18 years with FLT3mut+R/R AML who had been treated with 120 or 200 mg/d giltertinib. These doses were identified due to their ability to induce high clinical response rates, and consistent, potent FLT3 inhibition in in vitro and in vivo 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<sup>-2</sup> as the threshold for improved survival.

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

Summary/Conclusions: Molecular responses to giltertinib in FLT3-ITDmut+R/R AML correlated to 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 giltertinib therapy and validate FLT3 as a critical therapeutic target in AML.

S111

RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: RESULTS OF THE AML1310 TRIAL OF THE GIMEMA GROUP


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Background: A comprehensive AML risk assessment, based on the integration of cytogenetic/genetic data and minimal residual disease (MRD) status, can help optimize patients’ (pts) therapeutic post-validation assignment.

Aims: To evaluate the feasibility and results of a phase II trial of intensive chemotherapy in which risk-assignment and post-remission therapy of young patients with AML was based on pre-treatment cytogenetic/genetic data and post-consolidation levels of MRD.

Methods: Between January 2012 and May 2015, 515 pts with de novo AML, 18 to 60 years old, seen at 55 GIMEMA institutions were enrolled in the trial. Induction consisted of i.v. daunorubicin 50 mg/m<sup>2</sup> daily on days 1 and 3 and i.v. etoposide 50 mg/m<sup>2</sup> daily on days 1 to 5; i.v. cytarabine 100 mg/m<sup>2</sup> as a daily continuous infusion, days 1 to 10. All pts in CR/CRi after 1-2 induction cycles, received 1 consolidation course consisting of i.v. daunorubicin 50 mg/m<sup>2</sup> daily on days 1 to 6 and i.v. cytarabine 500 mg/m<sup>2</sup> every 12 hours on days 1 to 6. In pts belonging to ELN low or intermediate-risk category, peripheral blood stem cell collection was attempted by initiating, on day 20 from the start of consolidation therapy, G-CSF until completion of stem cell collection. Post-consolidation therapy was based on risk-allocation. Low-risk pts (NPM1 positive FLT3-ITD negative or CBF positive) were to receive AuSCT or ASCT depending on the source of stem cells (identical sibling, unrelated, cord blood, haplodiploidal). In pts, 500/515 pts started treatment and were available for the analysis. Median age was 49 (18-61) years and 52% were males. Of 429 evaluable pts, ELN cytogenetic distribution was: low-risk 11%, intermediate-risk 73% and poor-risk 15%. A RUNX1/RUNX1T1 was detected in 24% of all pts. Of 96 pts, CBFbeta/MYH11 in 7% of 496, FLT3-ITD in 25% of 497 and NPM1 in 37% of 499. In 494 evaluable pts, complete remission rate (CR) was 73% (361), 18% had refractory AML and 9% died early during induction. Three hundred-41 pts completed the consolidation phase and were risk allocated. 114 (33%) to the low-risk category (=AuSCT), 122 (38%) to the high-risk category (=ASCT), 223 (56%) to the intermediate category (=AuSCT or ASCT). In 27 pts (8%) belonging to the intermediate-risk category, a leukemia associated phenotype was not found and they were to receive AuSCT. Overall, 109 (33%) and 123 (36%) of 341 pts received AuSCT and ASCT, respectively. Median follow-up was 27.9 months. At 24 months overall (OS) and disease-free survival (DFS) of the whole series was 55.9% and 54.9%, respectively: cumulative incidence of relapse was 30.7%. At the same time point of 24 months, OS and DFS in the low-risk category was 79.8% and 63.8%, respectively; in the intermediate-risk category 40.5% and 44.8%, respectively; in the intermediate-risk category MRD negative 78.6% and 61.4%, respectively; in the intermediate-risk category MRD positive 69.8% and 66.6%, respectively (Figure 1).

Summary/Conclusions: A program of risk-adapted, MRD-driven therapy is feasible in a multicenter, cooperative setting. In the intermediate-risk category,
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.

S112

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

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**Background:** The detection of minimal residual disease (MRD) in patients with acute myeloid leukemia (AML) may improve future risk-adapted strategies of AML treatment. The presence of MRD 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=228), or conventional PRT by a third cycle of chemotherapy (n=160) or autologous HSCT (n=105). Endpoints of the study included overall survival (OS), relapse-free survival (RFS), and cumulative incidences of relapse and non-relapse mortality (NRM) at 4 years. A time-dependent covariate alloHSCT with the cumulative incidence of relapse was similarly distributed among MRD negative and MRD positive patients. No differences were present in transplant characteristics in MRD negative and MRD positive patients. OS and RFS was significantly better in patients without MRD (5-year OS: 5.89; 95% CI 3.32-10.47) and overall survival (HR 3.62; 95% CI 1.86-7.04) as compared to total amount of WBCs and LSC-positivity was defined as a proportion of LSC events measured).

**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±24% at 4 years, p<0.001, respectively), while NRM was not significantly different and estimated 10±1%. NRM split by EBMT risk classification 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 improving 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 CD34CD38- stem cell frequency and MRD frequencies to investigate impact on patient outcome.

**Methods:** In 242 patients who achieved morphologic complete remission, both LSC and MRD data after two cycles of chemotherapy treatment were available. MRD-positivity was defined as a percentage of MRD-positivity of above 1% (as compared to total amount of WBCs) and LSC-positivity was defined as a CD34CD38-LSC percentage above 0.0000% (LSC cut-off 0.0000%; thus no CD34CD38-LSC events measured).

**Results:** Cumulative incidence of relapse (CIR) and overall survival (OS) data were investigated for four different MRDLSC groups: 1. MRDpos+LSCneg-patients (n=136) 2. MRDpos+LSCneg-patients (n=28) 3. MRDneg+LSCpos-patients (n=58) and 4. MRDpos+LSCpos-patients (n=20). Results showed that MRDpos+LSCpos-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 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 MRDneg+LSCneg-patients and MRDneg+LSCpos-patients.

**Summary/Conclusions:** Overall, we conclude that our prospective results show that CD34CD38-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.

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


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

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

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

Results: MFC-MRD results after either induction course are available for 1555 patients randomised from 4/09-12/14 (median age 51, range 0-73). Cycle 1 (C1) response data with MFC-MRD was available for 1,400 patients. 70% achieved morphological CR at this time-point; 14% had resistant disease (RD) and 16% were in partial remission (PR) according to clinician. Of patients in CR (n=984) 56% had detectable MFC-MRD (MRD+). Excluding poor-risk patients 14% of patients did not achieve CR (7% RD, 7% PR), 51% of patients in CR were MRD+. 5 year OS for MRD- vs MRD+ was 63% vs 44% vs 44% vs 37% vs 25% for all patients; 69% vs 51% vs 50% vs 30% excluding poor-risk patients and 66% vs 49% vs 49% vs 30% for standard risk alone (Figure 1). The similar OS in this group between CR MRD+ and PR at C1 was maintained in NPM1/wt standard risk patients and if censored at stem cell transplant. 771 patients were in CR post cycle 2 (C2) and provided MFC-MRD data. As expected, there were significant differences in 5 year OS between CR MFC MRD- vs CR MFC MRD+ for all patients (35% vs 63%) and excluding poor-risk (38% vs 70%, n=512). Importantly post cycle 2 MFC-MRD status also differentiated OS for NPM1/wt standard risk patients with 5 year OS of 32% vs 64% (P=0.002) for MRD+ vs MRD- (Figures 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, Nadeau 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 be important 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 alterations (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-deep 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. CNA 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-gradual 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 CNA alterations allowed to detect at least one alteration in 66% of the patients. The mutational complexity (accumulation of 1 to ≥4 driver alterations), but not the presence of subclonal driver populations, gradually shortened the 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 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.

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

Aims: 2.5-4% of CLL patients harbor 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 chemotherapy 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.

Methods: FBXW7 mutations were identified in the PolyPhen-2 software. all except one next-generation sequencing in primary CD19-sorted samples of previously untreated CLL patients (n=905) as well as in CLL (n=8), MCL (n=5), Burkitt lymphoma (n=1) and LCL cell lines (n=3). In silico modeling with PolyPhen-2 predicted a potential impact of the mutations on the structure and function of FBXW7. FBXW7 mutant HG3 cell lines were compared to CRISPR/Cas9 in the CLL cell line HG3, which does not harbor a NOTCH1 mutation. Both in this CRISPR/Cas9 mutated cell line and in primary CLL cells with FBXW7 mutations, the protein levels of FBXW7 substrates were examined. In addition, we quantified NOTCH1 and HIF-1α activity with Luciferase reporter assay in FBXW7 mutated HG3 cell lines.

Results: Heterozygous mutations in FBXW7 were found in 41/905 (4.5%) of CLL patients. The most common mutations of FBXW7 were missense mutations (32/41) that target the substrate binding domain of the FBXW7 protein as well as nonsense mutations (4/41). Interestingly, 5 patients harbored two concurrent driver mutations. By the use of 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 CLL, MCL and LCL 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, HIF-1α- 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 wild-type 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 hence targets 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) transposase-accessible chromatin assays (ATAC-seq) and iv) whole-genome bisulfite 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 (Hi-C-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 (177 CLLs) and the active chromatin landscape (100 CLLs). CLL is closer to naive and 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 further strengthen the two major clinical subgroups of CLL with unmutated and mutated immunoglobulin heavy chains (IGHV). 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=489) or loss (n=38) 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 IGHV 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 lose poised promoters, which are replaced by repressive inactive 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, transcriptional, and 3D chromatin landscape in CLL. In addition we provided the community with an extensive resource of epigenetic information of this lymphoid neoplasm.

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**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 understudied.

**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 screened inhibitors of Bruton tyrosine kinase (BTK, ibrutinib), phosphoinositide-3 kinase (PI3K, idelalisib) and spleen tyrosine kinase (SYK, entospletinib). We quantified CLL cell apoptosis, migration, NFκB activity, protein and inositide-3 kinase (PI3K, idelalisib) and spleen tyrosine kinase (SYK, entospletinib). We supposed that SYK directly complexed with TRAF2/3 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.
H. Sakai1,2, N. Hosono1,3, H. Nakazawa2, B. Przychodzen1, C. Polprasert1, A. NOVEL GENETIC AND MORPHOLOGIC PHENOTYPE OF ARID2-DNA-repair pathways.

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 syndrome (MDS)-like disease in mouse models during ageing, indicating that MYBL2 could be acting as a tumour suppressor gene within del20q abnormality. Moreover, our previous work demonstrated that regardless of del20q deletion, MYBL2 expression is reduced in CD34+ bone marrow cells from MDS patients with worse prognosis. Because it has been shown that the cell of origin of MDS is the haematopoietic stem cell (HSC) and given the role of MYBL2 in DNA replication fork progression and maintenance of genome integrity, we hypothesised that low MYBL2 levels in HSC could contribute to elevated somatic mutations through changes in DNA repair pathways and drive disease development.

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

Methods: In this study we used our mouse model in which animals express ~50% normal levels of MYBL2 (Mybl2+/−). We characterised the ability of HSCs from young (7 weeks) and old (70 weeks) animals to respond to in vivo ionising radiation (2Gy) in terms of proliferation, apoptosis and colony forming ability. We measured the activation of the two main DNA repair pathways operating in the cells to deal with DSB: the error-prone 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 repair quite efficiently by NHEJ, but this efficiency is disrupted when cells are challenged with ionising radiation. Furthermore, Mybl2+/− HSCs have increased sensitivity to inhibition of DNA-PKc (required for NHEJ but not ATM (required by HR)). We also observed that after ionizing irradiation Mybl2+/− HSC progeny displayed an increased percentage of chromatids with fragile telomeres. Moreover, by making use of publically available RNA-seq datasets from MDS cases, 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 DSB repair in HSCs and suggests that low levels of MYBL2 in human MDS could contribute to the emergence of further genetic abnormalities by deregulation of DNA-repair pathways.

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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 previously reported, but many other rare 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 detect ARID2 mutations in a variety of solid tumors.

Aims: 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 splicing mutations in ARID2 missplicing and gene expression.

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 yielded defective ARID2 transcripts. Clone 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 homozygous 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 glycoprotein 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.

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.

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THE VALUE OF NGS PANEL SEQUENCING TO MOLECULARLY DEFINE MEYLOID MALIGNANCIES AND CLARIFY BORDERLINE CASES: A STUDY ON 39 GENES IN 1143 PATIENTS

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Background: The myelodysplastic syndromes (MDS) are disorders of the hematopoietic stem cell (HSC) and patients suffer from anemia and other cytopenias and show increasing bone marrow blasts over time. Mutations in splicing genes (including SFB1, SRSF2 and U2AF1) occur in >50% of MDS patients.

Aims: To identify the deregulated pathways and gene ontology (GO) categories associated with aberrantly spliced genes in CD34+ cells and in different cell types of MDS-affected lineages isolated from the bone marrow of patients with MDS, harboring mutations in splicing genes.

Methods: Transcriptome data were generated using RNA sequencing (RNA-seq) and splicing factor mutant cases were compared to wildtype cases and to healthy controls. (aberrant) splicing events were associated with each mutated splicing factor tended to affect different sets of genes, although some overlap was observed. The most frequently mutated genes were associated with each mutant splicing factor gene (in the comparison to both healthy controls (18 of 30 in the comparison to wildtype cases) were common to all three mutated splicing factor genes. Pathway analysis revealed deregulated pathways associated with 'oxidative phosphorylation' and 'mitochondrial dysfunction' that were common to more than one mutant gene (i.e. SF3B1 and SRSF2), and pathways specific for one mutated splicing factor gene (e.g. protein ubiquitination). We identified upstream transcriptional regulators showed a significant overlap between the aberrantly spliced genes identified by each splicing factor gene (in the comparison to both healthy controls and deregulated pathways and GO themes in cells of different lineages. There were many aberrantly spliced genes in one cell population that did not overlap with aberrantly spliced genes in other populations. A small proportion (i.e. ≤5%) of aberrantly spliced genes were common to all cell populations. GO analysis of the aberrantly spliced genes identified showed that 6 of the top 30 most significant categories (including RNA-binding and RNA metabolism) were common to all 4 cell populations. The most significant GO categories (4 of 30 in the comparison to wildtype cases) were common to all four cell populations studied. Pathway analysis revealed that several pathways were deregulated in specific cell populations (e.g. mTOR signaling in erythroid cells), and some pathways (e.g. EIF2 signaling, involved in protein synthesis initiation) were upregulated in all four cell populations studied.

Summary/Conclusions: Our study has identified aberrantly spliced genes and deregulated pathways associated with splicing mutations in the HSCs and the major cell lineages affected in MDS, providing new insights into how these mutations impact cellular processes in this disorder.

S122

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

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Methods: We performed transcriptome sequencing of bone marrow mononuclear cells (BMMNCs) and/or CD34+ cells obtained from patients with myelodysplasia. Consensus clustering was used to identify stable patient clusters. A classifier of the gene expression-based subgroups was constructed using the 100 CD34+ cell samples as a training set, followed by validation in an independent cohort of 183 MDS patients. Another classifier was constructed using BMMNC samples from 51 patients, who had been assigned to the subgroups by the gene expression data of their CD34+ cells. Prognostic significance of the model was tested in 114 patients of myelodysplasia.

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

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

Lymphoma biology

S124

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

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

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

Methods: SVs and other genetic lesions affecting PD-L1 and PD-L2 were analyzed using targeted-capture sequencing with crRNA baits designed for capturing the entire sequences of PD-L1 and PD-L2 genes, including exons, introns, and 5'- and 3'UTRs. More than 400 samples were analyzed obtained from different subtypes of non-Hodgkin lymphomas, including EBV-associated lymphomas, such as EBV-positive diffuse large B-cell lymphoma (DLBCL) and NK/T-cell malignancies.

Results: SVs and/or focal copy number gains involving PD-L1 were successfully detected in various B-cell and T/NK-cell lymphomas, albeit at generally low frequencies (<10%). These lesions were the most frequently observed in PBMCs, accounting for more than 60% of the cases. High frequency (17–57%) of PD-L1/PD-L2-involving abnormalities were observed in mature NK/T-cell neoplasms, including extranodal NK/T-cell lymphoma, aggressive NK cell leukemia, and EBV-positive T-cell lymphoproliferative disorder, all of which were positive for EBV. Moreover, a substantial proportion (22%) of EBV-positive DLBCL cases possessed these lesions, whereas EBV-negative cases rarely exhibited these alterations (2%, P<0.01). For both PD-L1 and PD-L2 SVs, despite a large diversity of SV type (deletions, inversions, tandem duplications, and translocations), most of SVs resulted in 3'-UTR truncation, while the replacement of PD-L1 or PD-L2 promoter with an ecotropic regulatory element was rarely observed. Interestingly, PD-L1 SVs were detected in both B- and T-cell lymphomas, whereas PD-L2 SVs were found exclusively in B-cell lymphomas.

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

S125

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

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Background: Recurrent somatic mutations of N-terminal region of FOXO1,
shown previously to increase 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 mechanisms 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 downregulate 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. To determine the influence of tumors on cellular interactions between FOXO1 and CD20 promoter we performed EMSA and ChIP experiments. For animal studies we used SCID Fox Chase mice model. All in vivo 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 in CD20 protein in regulation, we disrupted FOXO1 focus using the CRISPR/Cas9 genome editing technology in Raji cells. In 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 transcription could be translated into tumor efficacy in vivo we have used SCID Fox Chase mice model. We found that mice treated with systemic rituximab survived longer when inoculated with sgcFOXO1-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 regulated the CD20 promoter activity. In different B-cell lymphoma cell lines MK-2206 and GDC-0068 significantly downregulated the levels of MS4A4F transcript (encoding CD20). Finally, using both EMSA and ChIP assays we detected specific binding of FOXO1 to the MS4A4F 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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THERAPEUTIC INTERVENTION.

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

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

**Methods:** Primary Nipa<sup>-/-</sup>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 Ba/F3 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 immunophenotype and clinical presentation.

**Results:** Primary Nipa<sup>-/-</sup>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 Ba/F3 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-Cre<sup>TG<sup>129Vfp<sup>/<sup>MSNAIE infected bone marrow cells showed significantly prolonged survival (mean survival 141d vs 80d in wt). Morphologically, mice presented with enlarged thymi, splenomegaly, lymphadenopathy, and bone marrow infiltration. Immunophenotyping showed a pure T-cell phenotype in Nipa<sup>-/-</sup> 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) in mice transplanted with M<sup>i</sup>g<sup>tm</sup>NPM-ALK/Nipa-<sup>-/-</sup> bone marrow compared to control animals were observed, thereby suggesting a crucial role of NIPA in NPM-ALK driven lymphomagenesis. To investigate the precise mechanism underlying these results, we performed cell cycle analyses as well as cell viability assays. Indeed, we were able to detect significant differences in the cell viability in 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.

**Thalassemia**

**S128**

**GENE THERAPY FOR BETA THALASSEMIA: INITIAL RESULTS FROM THE PHASE III TIGET-BTHAL TRIAL OF AUTOLOGOUS HEMATOPOIETIC STEM-CELLS GENETICALLY MODIFIED WITH GLOBE LENTIVIRAL VECTOR**

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

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

**Methods:** On the basis of extensive efficacy and safety preclinical studies, the clinical trial TIGET-BTHAL was approved and started in 2015 at Scientific Institute Tute 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 (β<sup>0</sup>, β<sup>−</sup> and β<sup>+</sup>) have been treated with GLOBE-transduced CD34<sup>+</sup> cells at a dose of 16x10<sup>6</sup>-19x10<sup>6</sup> 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 B-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 (Suragani R, Blood, 2015) and increased Hgb expression 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/8 weeks prior to first dose, titration up to 1.25 mg/kg). Pts in the expansion cohort and those who rolled over to the ext phase 2a clinical trial. Main inclusion criteria included adult patients (>30 years of age), with β-thalassemia major (TM) with a complex pathophysiology. We have previously shown that RANKL, the most potent osteoclast activator, is elevated in the serum of TM patients (TM) with 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 TRANSPANTATION FOR THALASSEMAIA: THE FRENCH EXPERIENCE


Background: In clinical practice, allogeneic hematopoietic stem cell transplantation (H SCT) is the only treatment offering a definitive cure for patients with beta-thalassemia. Its outcome has improved over the last 3 decades with the introduction of new HCT regimens and conditioning regimens. The purpose of the study was to evaluate the long-term health status after a successful allogeneic H SCT for beta-thalassemia major in a national cohort of patients.

Methods: This French retrospective study included patients who successfully received allogeneic H SCT between 1985-2012 and were alive at least 2 years after H SCT. Study was based on data collected in the national registry of patients with beta-thalassemia and conducted in collaboration with the French Society of Hematology and the French Hematology Society (SFGM-TC). Late effect data were recorded by physicians through reference or transplant center visits. Collected data included medical examination results, long-term treatments administered and laboratory tests (serum ferritin, Hb, liver enzymes, creatinine level and thyroid evaluation). Linear mixed-model was used to analyze data evolution over time (for height and weight SDS, SF, Hb values).

Results: A total of 134 patients had received allogeneic H SCT for beta-thalassemia in France from 1985 to 2012. 107/134 patients experienced successful H SCT (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 other causes. For 119 patients were analyzed for long-term effects. Median age at H SCT 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 hypothyroidism, 2 heart failure, 5 diabetes. 2 patients had chronic respiratory failure related to transplant. The height SDS improved after H SCT if performed at a young age. Weight
SDS values increased with time, especially in females. Although gonadal dysfunction was observed in 60% of women aged at least 13 years at the last evaluation, 12/27 aged more than 20 years experimented at least one successful pregnancy. 93 patients had stopped their immunosuppressive treatment two years after HSCT. 37 were treated with iron chelation therapy and/or phlebotomies. At least half of patients are receiving a long-term hormonal treatment or antibiotic prophylaxis at the last visit. Decrease in serum ferritin values after transplant was significantly influenced by age at transplant and pre-transplant serum ferritin value. The median hemoglobin value was 12.5 g/dL (8.6-165) at a mean age of 18 years and Hb values were significantly influenced by age, the sex of the donor and the presence of donor thalassemia trait.

Summary/Conclusions: A comprehensive and regular long-term follow-up should be established for all patients receiving allogeneic HSCT for beta-thalassemia major. In this national cohort, endocrinological complications were frequent after transplant. Fertility can be partly preserved, but this result has to be reevaluated with the more recent use of intravenous busulfan.

S132

CD34+AND HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION TO TRANSFUSION READY RED BLOOD CELLS
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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 (rRBC), 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)×1012 erythrocytes, and for iPSC we need virus- and transgene-free reprogramming protocols. To solve the above mentioned issues a customized humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1×1012 times erythropoiesis from iPSCs to pure adult EBL cultures within 25 days, comparable to non-GMP commercial media. To generate iPSC, a non-integrative polyclonstronic episcellular vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was used to reprogram PBMC-expanded EBLs to iPSC, displaying pluripotency potential and normal karyotype. iPSCs were adapted to single cell passage allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth media, allowing directed colony differentiation using a feeder-free monolayer approach.

Summary/Conclusions: Here we showed that our monolayer approach is simple, highly controlled and compatible with upsamping. Avoiding virus-, integrative reprogramming, feeders and with our GMP-grade media we maintained a cost effective system moving toward clinical application. Further maturation of iPSC-EBLs from 1200iPSCs within 21 days (12

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 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-EEN, MLL-GAS7 and MLL-AF1p) were purified from stable cell lines allowing for inducible, single-copy transfection. Expression and characterization by mass spectrometry. Data analysis identified a comprehensive protein-protein interaction network, which was functionally interrogated by a subtractive shRNA screening approach. Valudation 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 nuclease by 7 MLL fusion proteins by affinity purification coupled to mass spectrometry (AP-MS) revealed a densely interconnected protein-protein interaction network of 963 proteins, comprising previously known MLL-interacting protein complexes (such as PRC2 or SWI/SNF), as well as a high number of new interaction partners of MLL. 128 proteins were found to interact with ≥5 of all 7 MLL-fusions. This subset of conserved MLL-interaction partners was highly enriched for proteins with function in chromatin metabolism and transcriptional control. Systematic functional investigation of the conserved MLL-fusion interactome using subtractive shRNA screens identified the methyltransferase SETD2 as a critical effector of MLL fusion proteins. Both RNAi-based suppression and CRISPR/Cas9-mediated mutagenesis of SETD2 induced myeloid differentiation and apoptosis in human and mouse MLL-rearranged cell lines, while having only modest effects on the proliferation of MLL-wild-type leukemia cells. Depletion of SETD2 in MLL-fusion-t contracts for changes in proliferation, differentiation, apoptosis and DNA damage. SETD2 is a critical effector of MLL fusion proteins.

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 CEBPα mutants 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 Cebpa+/−MLL mouse model. Cellular competition assays were used to assess changes in proliferative capacity and clonogenicity. Further, MTT (metabolic tetrazolium) 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 Menin promoter. Further, we showed that C/EBPα functions as a transcriptional repressor of Menin. To test the hypothesis that small molecules can specifically inhibit C/EBPα, we introduced targeted mutations across the Mll gene in Cebpa+/− MLL cells using the CRISPR/Cas9 system. This analysis revealed a strong dependence of Cebpa+/− MLL cells on the expression of an intact MLL protein. Surprisingly, loss of the enzymatic activity of Mll by mutational targeting of the SET domain did not significantly affect cell survival. In contrast, cells were particularly sensitive to mutations of the Menin-binding motif in MLL. Mll targeting strongly induced myeloid differentiation in Cebpa+/− MLL cells as measured by increased levels of myeloid surface markers. To test the functional impact of our findings, 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 Cebpa+/− MLL cells. RNA-seq analysis revealed that treatment induced 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 Cebpa+/− MLL cells were 2-6 fold more sensitive than other leukemia cell lines of mouse and human origin.

Summary/Conclusions: We show that C/EBPα-mutated 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

Aims: We hypothesized that the interaction between the oncogenic C/EBPα and 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 repopulating capacity of PU.1lowAML 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. Gene expression analysis showed significant downregulation of PU.1 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. Together, these findings indicate that the compounds significantly decreased tumor burden and increased survival.

Summary/Conclusions: Our study describes 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 (FLT3mut) are amongst the most frequent in AML and are associated with a poor outcome. FLT3mut promotes constitutive activation of survival/proliferation pathways and resistance to FLT3 TK inhibitors (TKI). FLT3mut has been shown to lead to changes in cellular metabolism, including increased glycolysis. The FLT3 TK represents a valid therapeutic target and several FLT3 TK inhibitors (TKI) have been developed. However, despite showing activity in the preclinical setting, FLT3 TKI have displayed limited efficacy in clinical trials. Resistance mechanisms to FLT3 TKI include receptor mutations and cell intrinsic adaptive mechanisms. Amongst the latter, metabolic adaptations 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 FLT3mut-AML, following TKI treatment, in an attempt to unveil novel therapeutic vulnerabilities.

Methods: Liquid chromatography coupled to mass spectrometry (LC/MS), using stable isotope-based carbon flux tracing, and oxygen consumption rate/extracellular acidification rate as measured by an extracellular flux analyser (Seahorse, Agilent Technologies) were used to assess metabolic changes in FLT3mut-AML cells. Gene expression was measured using qPCR and enzyme activity was measured by flow cytometry. 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 FLT3mut compared to FLT3 wild-type (FLT3wt) patient samples at diagnosis. We then confirmed that both human and murine FLT3mut cells display increased glycolytic and respiratory capacity compared to FLT3wt cells. Furthermore, FLT3mut cells exhibit increased glycolytic, citric acid cycle, and oxidative phosphorylation genes as 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.

Summary: FLT3 mutation results in metabolic reprogramming, with increased glycolysis and decreased oxidative phosphorylation, that may be targeted to improve TKI efficacy.
FLT3 TK activity may improve the eradication of FLT3mut AML cells. Glutamine metabolism is mostly channelled towards glutathione production, and sustaining macromolecule biosynthesis and cellular energetics. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK activity may improve the eradication of FLT3mut AML cells.

Summary/Conclusions: Our data suggest that upon AC220 treatment, glutamine metabolism becomes a critical metabolic dependency in FLT3mut AML. 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.

Hematopoiesis, stem cells and microenvironment

S137
STEP-WISE REPROGRAMMING OF ENDOTHELIAL CELLS INTO IMMUNE-COMPETENT HEMATOPOIETIC STEM CELLS
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Background: The molecular pathways and microenvironmental cues that choreograph the conversion of endothelial cells (ECs) into long-term repopulating hematopoietic stem cells (HSCs) remain poorly defined. This is due to lack of models that recreate the ephemeral transition of an endothelial cell to a hemogenic state to the emergence of HSCs.

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

Methods: Here, we have developed a modular in vitro model in which—by precise, conditional expression of transcription factors: FosB, Gfi1, Runx1, and 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+) resulted in 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.

S138
MARROW MESENCHYMAL STEM CELLS RESCUE BONE MARROW ENDOTHELIAL CELLS SUFFERING CHEMOTHERAPY STRESS BY TRANSFERRING MITOCHONDRIA THROUGH NANOTUBES
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Background: The tunneling nanotube (TNT) is a newly discovered, long and thin tubular structure between cells and can facilitate the intercellular exchange of diverse cellular signals and components ranging from electrical signalling to organelles. Recent reports show that mesenchymal stem cells (MSC) rescue injured target cell and promote target cell recovery from a variety of stress including oxidative tress, ultraviolet radiation, ischemia/reperfusion (I/R)et al. 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 (BMD EC) and promote it recovery from chemotherapy stress, etc. 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 BMD ECs or HUVEC, illuminating its constituent and investigating the significan of transport of mitochondrial through TNT between BM MSC and BMD ECs or HUVEC suffering from chemotherapy stress of cytotoxic 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 (BMD ECs) or Human umbilical cord vein endothelial cells (HUVECs) respectively.

Results: Firstly, We observed the TNTs formed between BM MSCs and endothelial cells including the TNTs structure between BM MSCs and HUVECs or BMD ECs are composed with F-actin, microtubule in addition to membrane. Live cell imaging showed the two xeno-genetic cells form TNTs by retaining a thin thread of membrane upon distodge.
SHORT-TERM FEEDING OF A HIGH-FAT DIET DISTURBS LIPID RAFT/TGF-BETA SIGNALING-MEDIATED QUIESENCE 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 intracellular 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 a high-fat diet (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 observed impact was obtained 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.

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Gene therapy, immunotherapy and vaccination 1

S141
WILMS’ TUMOR 1 RNA- ELECTROPORTATED 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-remission 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 their own DC1 (WT1) antigen by means of mRNA electroporation, a technique that allows for human leukocyte antigen haplotype-independent, multi-epitope antigen presentation to T-cells. The vaccines were administered intradermally. WT1 mRNA levels in blood and marrow were followed as a measure of minimal residual disease. Circulating WT1-specific CD8+ T-cells obtained before vaccination and after the 4th dose were stained with WT1-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 restimulated 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 CR1 to CR2 after DC vaccination by DC1 vaccination only. In the remaining 4 responding patients, the clinical response was characterized by stable disease as demonstrated by elevated but stable WT1 transcript levels in blood for 3-12 months and stable blood values without blasts. Five-year overall survival was 40%, as compared to 24.7% in the SEER data of the National Cancer Institute; it was significantly higher in responders than in non-responders (53.8% vs 25.0%; P<0.01). In patients receiving DCs in first complete remission (CR1), there was a vaccine-induced relapse reduction rate of 25% and the 5-year relapse-free survival was significantly higher in responders than in non-responders (50% vs 7.7%; P=0.0001). In patients ≥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, 15 in CR1, 3 in CR2, and 15 progressed to relapse. The clinical and blood response was correlated with WT1 mRNA levels in blood and marrow and with the presence of WT1-specific CD8+ T-cells. Treatment was generally well tolerated and adverse events were moderate.

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. Long-term clinical response was correlated with vaccine-induced WT1-specific CD8+ T-cell responses.

S142
FIRST-IN-HUMAN MULTICENTER STUDY OF BB2121 ANTI-BCMA CAR T CELL VACCINATION IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS
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Aims: To test the safety and efficacy of the CAR T cell modality in relapsed/refractory multiple myeloma (MM), we have designed a second-generation CAR construct targeting B cell maturation antigen (BCMA) to redirect T cells to MM. bb2121 consists of autologous T cells transduced with a lentiviral vector encoding a novel CAR incorporating an anti-BCMA scFv, a 4-1BB costimulatory motif and a CD3-zeta T cell activation domain. We will report updated safety and efficacy results following promising initial results (Berdeja et al. ENA 2016).

Methods: CRB-401 (NCT02658992) 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. There were no prior CAR therapy, double-refractory MM, ≥50% BCMA expression in bone marrow plasma cells. Peripheral blood mononuclear cells are collected via leukapheresis. Patients undergo lymphodepletion with Flu (30 mg/m2) Cy (300 mg/m2) daily for 3 days then receive 1 infusion of bb2121. The study follows a standard 3+3 design with planned dose levels of 5.0, 15.0, 45.0, 80.0 and 120 x 107CAR+T cells.

Results: As of November 18, 2016, 11 patients had been infused with bb2121 in the first 4 dose cohorts, and 9 patients had reached at least 1 month of follow-up. As of data cut-off, no dose limiting toxicities, and no >Grade 2 neurotoxicities or cytokine release syndrome (CRS) had been observed. Grade 1-2 CRS has been reported in 8/11 (73%) treated patients. All patients treated with dose level 5.0 x 107 or higher had ≥ Grade 1 CRS. The patients in the evaluable group consisted of 9 patients, including 8 responders and 1 non-responder. The ORR was 100%, including 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.

S143
BASELINE AND EARLY POST-TREATMENT CLINICAL AND LABORATORY PARAMETERS ASSOCIATED WITH SEVERE NEUROTOXICITY FOLLOWING 19-28z CAR T CELLS IN ADULT PATIENTS WITH RELAPSED B-ALL
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Aims: We examined baseline and post-treatment clinical and laboratory parameters to identify factors associated with severe Ntx (≥ Grade 3) in our phase I clinical trial of CD19-specific 19-28z CAR T cells for adult patients (pts) with R/R B-ALL (NCT01044069).

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

Results: In this cohort of ALL pts, 20, 8, 2, 18 and 3 pts experienced Gr 0, 1, 2, 3, and 4 NTX, respectively. No pt developed grade 5 NTX and no cerebral edema was seen. Disease burden (≥50% blasts) at the time of T cell infusion (p=0.0045) and post-treatment sGR3 CRS (p=0.0010) were significantly associated with sNTX, but we found no association with age, weight, T cell dose, choice of conditioning chemotherapy (Flu/Cy vs S Cy), and prior lines of treatment. Among the clinical and blood parameters, fever, low PLT, high ferritin and MCHC as well as elevated GM-CSF, IFNγ, IL-5, IL-5, IL-10, IL-2 at day 3 of T cell infusion at day 3 of T cell infusion were significantly associated with sNTX (all p<0.01). While some of these cytokines were also elevated in severe CRS cases, IL-5 and IL-10 at 2 days are unique to sNTX. Furthermore, in vivo peak CAR T expansion at day 7 (p=0.001) significantly correlated with sNTX (p<0.01). Lastly, multivariate analysis revealed baseline PLT ≤50 or MCHC ≤33.3% and morphologic disease (≥5% blasts) has 95% sensitivity and 70% specificity of identifying sNTX pts.

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

S144
FIRST EVIDENCE DEMONSTRATING ENGRAFTMENT AND RESPOPULATION ADVANTAGE OF GENE-CORRECTED HEMATOPOIETIC REPOPULATING CELL IN NON-CONDITIONED FANCONI ANEMIA PATIENTS
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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 corrected HSCs.

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

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

Results: Eight patients have been included so far in the HSC collection trial. No severe adverse events (SAE) related to the procedure have been reported. The most relevant AE has been the transfusion of packed red blood cells and platelets. Six FA patients aged 3-6 years underwent collections after mobilization of significant numbers of CD34+ cells (10 to 70 CD34+ cells/µl) to peripheral blood. Two patients (15 and 16 years) failed to mobilize. On average, 5 million CD34+ cells/Kg were collected, with 45% recovery after immunoselection. In the first patient included in the gene therapy trial, fresh immunoselected CD34+cells were transduced with the therapeutic vector. Subsequently, two patients were infused with transduced CD34+ cells that remained cryopreserved for almost 2 years. Infused cell products contained 0.5 to 1.4 million CD34+ cells/kg, and vector copy numbers per cell (VCN/cell) that ranged between 0.17 to 0.45. To date, there has been no SAE related to the procedure. Engraftment of gene corrected cells has been observed in the three patients. Notably, increased gene marking levels and significant phenotypic correction 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 corrected HSCs.

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

S145

TARGETING FLT3 WITH CHIMERIC ANTIGEN RECEPTOR T CELLS CONFRSES 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, a receptor 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 morphologic 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 5x106 CAR-modified or control T cells (CD8/CD4 ratio=1:1).

Results: We confirmed specific recognition and high-level cytolytic activity of CD8+FLT3-CAR T cells against a panel of AML cell lines including THP-1 (FLT3 wild type), and Molm-13 (FLT3-ITD heterozygous). Both CD8+ and CD4+ FLT3-CAR T cells produced IFN-γ and IL-2, and underwent proliferation after antigen stimulation. FLT3-CAR T cells that we prepared from AML patients exerted specific anti-leukemia reactivity against autologous primary AML blasts, with near-complete cytolytic evasion 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 allo-genic HSC transplantation. 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.

22nd Congress of the European Hematology Association
Background: Allogeneic hematopoietic stem cell transplantation (HSCT) offers curative therapy for children who lack an available HLA-identical donor with hematopoietic disorders such as Primary Immune Disorders (PIDs), hemoglobinopathies, erythroid disorders and acute leukemias. \( \frac{\text{CD3+/CD19+ T-cells were detectable at one year via flow cytometry analysis}}{\text{CD4, CD8 (Figure 2B) and B cells (Figure 3C) immune reconstitution was brisk.}} \]

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 \( \frac{\text{αβ T-cell depleted haplo-HSCT}}{\text{Patients were infused with BPX-501 T cells 2 weeks post-transplantation. 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=65</th>
<th>Malignant</th>
<th>N=38</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD</td>
<td>11</td>
<td>ALL (CR1 CR2 CR3)</td>
<td>25</td>
</tr>
<tr>
<td>WAS</td>
<td>6</td>
<td>AMI</td>
<td>14</td>
</tr>
<tr>
<td>CD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalassemia Major</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sicca Cell Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fanconia Anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLI</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results: Cumulative incidence of TRM remains very low at 100 days (0%), 180 days (1.6%) and 1 year (2.8%). Of the 81 patients with >180 day follow-up, 20 patients had acute GvHD 1-3 (24.7%) (Figure 1A); 10 with Grade 1, 8 with Grade 2, 2 with Grade 3 and one Grade 4 skin. Mild cGvHD was seen in 2 patients, moderate cGvHD in 2 patients and one case of severe cGvHD in a malignant patient. attributing to the allograft, not BPX-501. Rinucidum was used in 4 patients with Grade 2 GvHD with rapid resolution of symptoms, as it did in the severe cGvHD patient. In both malignant and non-malignant patients. CD3, CD4, CD8 (Figure 2B) and B cells (Figure 3C) immune reconstitution was brisk. CD3+/CD19+ T-cells were detectable at one year via flow cytometry analysis of peripheral blood. In Wiskott-Aldrich patients, platelet recovery remains in the normal range at 180 days with mean platelet counts of 246.3±10x10⁴/μL. At 180 days and 1 year, the patients with hemoglobinopathies remain transfusion-free with a normal mean Hgb value of 11.4±g/dL.

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

S147

RE-CREATING HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN WITH CRISPR/Cas9 TO TREAT SICKLE CELL DISEASE AND BETA-TALASSEMIA

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

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

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

Results: We first optimized cell culture and electroporation conditions that led to high rates of genomic editing across multiple loci, achieving 84.9±6.2% (Mean±SD) editing efficiency at key regions of interest in CD34⁺ HSPCs from mobilized peripheral blood of healthy donors (n=16). Similar rates of editing were attained using CD34⁺ HSPCs derived from healthy-donor bone marrow (n=8). Cas9 delivery as recombinant protein improved cell viability when compared to mRNA-based delivery (98.8±3.7% compared to 75.5±9.3%, Mean±SD, n=56 for each) with no observed reduction in editing efficiencies. To investigate gene editing impact on HbF, edited cells were erythroid differentiated from healthy donors as well as SCD and β-Thal patients. Specific gene edits significantly increased γ-globin mRNA expression to therapeutically-relevant levels (increased expression to 29-37% as a ratio of γ/β in one β-Thal patient sample and to 25-45% as a ratio of γ/(γ+β) in six SCD patient samples). We demonstrated similarly high rates of editing in the CD34⁺/CD38⁺/CD45RA⁻ long-term repopulating HSPCs and bulk CD34⁺ HSPCs (87.9±6.4% compared to 89.7±6.9%, Mean±SD, n=56 for each). We confirmed that engraftment levels of edited cells in immunocompromised mice were similar to control cells (% human CD45 in peripheral blood = 28.6±9.9% in controls versus 27.1±6.6% and 26.3±7.9% for two guide targets, Mean±SD, n=48 for each). In-depth off-target analysis.

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for a selected guide RNA confirmed no detectable genomic cleavage at over 5000 predicted off-target sites with a detection sensitivity of 0.2%, supporting its safety for clinical use. Finally, we have demonstrated editing rates of >85% at clinical scale in a GMP-capable manufacturing facility to enable clinical development for SCD and β-Thal. Required safety toxicology studies are ongoing.

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

S148
EXPOSURE TO INFECTION TRIGGERS PAX5 AND ETV6-RUNX1 CHILDHOOD B-ALL
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1Experimental Therapeutics and Translational Oncology Program, Instituto de Biología Molecular y Celular del Cáncer, CSIC/ Universidad de Salamanca and Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain, 2Department of Pediatric Oncology, Hematology and Clinical Immunology, Heinrich Heine University, Düsseldorf, Germany, 3Department of Pediatric Oncology, Hematology and Clinical Immunology, University Medical Center, Amsterdam, Netherlands, 4Department of Hematology, University Hospital of Essen, Essen, Germany

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 infection triggers BCP-ALL subtypes that can be prevented 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 ensure Scal1-directed expression of BCR-ABL1p190 or ETV6-RUNX1 in HSC/PC and BCP-ALL subtypes can be induced by infection and how the pre-leukemic clone evolves to BCP-ALL.

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Summary/Conclusions: By analyzing the survival of xenotransfused human platelets 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 Rhoad-deficient platelets. The effect of G04 is reversible since removal of G04 after 7-day storage restores Rh0A activity to normal levels and allows normal extent of shape change and spreading on fibrinogen. To analyze the kinetics and hemo-mechanical changes of cold-stored Rh0A-deficient platelets after xenotransfusion, we analyzed the survival of xenotransfused human platelets after long-term (7-day) refrigeration in the presence and absence of inhibitors cocktail or individual inhibitors in macrophage-depleted, sub-lethally irradiated NSG mice (N=20/group) as well as autologously transfused platelets in a crossover trial in Rhesus monkeys (n=5). Our results show that reversible inhibition of Rh0A 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. Rh0A inhibition blocks the process of intracellular traffic of GP through lipid rafts and endocytotic intermediates as assessed by confocal microscopy of Gpib and the vascular selective protein VPS33b, as well as biochemical fractionation of detergent-insoluble membrane lipid rafts. Furthermore, we demonstrated that 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, NSC23766 and Casin, specific inhibitors for RhoA, Rac1 and Cdc42, respectively, were titrated and used at concentrations of 75 mcM, 50 mcM and 10 mcM, respectively. Rh0A-deficient murine platelets were obtained from polv1-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/mouse platelets were stored for 7 days at 4°C in plasma or P depends (67%/plasma/33%) at RT or 1°C for 7 days or 1-4 hours for murine platelets.

Results: We found that either short- or long-term refrigeration activates Rh0A and Rac1, but not CDC42. Genetic deletion of RhoA or RHODA 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 Rh0A-deficient platelets. The effect of G04 is reversible since removal of G04 after 7-day storage restores RHODA activity to normal levels and allows normal extent of shape change and spreading on fibrinogen. To analyze the kinetics and hemo-mechanical changes of cold-stored Rh0A-deficient platelets after xenotransfusion, we analyzed the survival of xenotransfused human platelets after long-term (7-day) refrigeration in the presence and absence of inhibitors cocktail or individual inhibitors in macrophage-depleted, sub-lethally irradiated NSG mice (N=20/group) as well as autologously transfused platelets in a crossover trial in Rhesus monkeys (n=5). Our results show that reversible inhibition of Rh0A 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. Rh0A inhibition blocks the process of intracellular traffic of GP through lipid rafts and endocytotic intermediates as assessed by confocal microscopy of Gpib and the vascular selective protein VPS33b, as well as biochemical fractionation of detergent-insoluble membrane lipid rafts. Furthermore, we demonstrated that platelet storage for transfusion uses room temperature and associates with a relatively high risk of bacterial growth and infection in susceptible patients.

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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 40 (8%) patients, 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

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TARGETED SINGLE CELL SEQUENCING TO IDENTIFY MUTATIONAL HIERARCHY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Acute lymphoblastic leukemia (ALL) is a common childhood malignancy caused by clonal proliferation of immature B or T lymphoid cells. ALL patients are primarily young children who respond well to chemotherapy, with survival rates above 85%. However, if relapse develops, survival rates drop to 15-50%. Recent studies have shown that at diagnosis, different ALL subtypes exist with mutations 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 collected using flow cytometry or a microfluidic device and analyzed with targeted sequencing. Cells were discarded from further analysis if focus and allele 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 mutations, while highly mutated HSPCs are eradicated during treatment, which is in line with long term remission in T-ALL.

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BCL-2 INHIBITION AS NEW THERAPEUTIC OPPORTUNITY FOR RPL10 R98S MUTANT PEDIATRIC T-ALL
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1Oncology, KU Leuven, 2Leuven Cancer Institute, Pediatric Oncology & Hematology, Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium

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 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: (i) Explore the oncogenic contribution of the RPL10 R98S mutation in pediatric T-ALL. (ii) Define new therapeutic opportunities for RPL10 R98S defective T-ALL. (iii) 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 with western blotting (western blotting) 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 Dihydroethidium and mitotracker. Viable cell counts were determined by Annexin V exclusion. Chromatin immunoprecipitation was performed using the Imprint ChIP 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 PPARγ and which were consequently upregulated together with CPT1A. Peroxisomal hyperactivation causes high intracellular H2O2 levels, maintaining 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 PPARγ 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 with the engraftment 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.

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 mutations, while highly mutated HSPCs are eradicated during treatment, which is in line with long term remission in T-ALL.

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

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

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Background: We previously described a recurrent arginine-to-serine mutation on residue 98 (R98S) in ribosomal protein L10 (RPL10), with a frequency of 8.6% in pediatric T-ALL cases. The R98S mutated residue contacts the catalytic core (peptidyltransferase center, PTC) of the ribosome and causes ribosome biogenesis, mRNA translation, and fidelity defects at the protein and cell levels. 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 the mutation and potentially driving oncogenicity.

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

Results: RPL10 R98S cells showed significant upregulation for 3% (n=178) of the measured proteins and a downregulation of 1% (n=68). Moreover, polysomal 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 those, 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 Ilcz2, a master regulator of the upregulated transcripts, was overexpressed at the transcriptional and protein level. Interestingly, the results from the mass spectrometry and the polysomal RNA sequencing datasets showed a significant enrichment and upregulation of members of the JAK-STAT signaling pathway.

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

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

Methods: 3070 approved drugs and pharmacologically active compounds were initially screened against an infant ALL cell line with MLL-rearrangement, PERR-455 and a paediatric leukaemia cell line wild-type for MLL, CEM, using a resazurin-based cell viability assay. Hit compounds were further tested in a panel of 10 paediatric leukaemia cell lines and 2 normal 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. Nrfl2 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 developed for rheumatoid arthritis and was later fast-tracked into Phase II clinical trial for adult chronic lymphocytic leukaemia, while Disulfiram, which was developed for treatment of chronic alcoholism, is currently in several clinical trials for cancers including metastatic melanoma and glioblastoma. These drugs also showed potent activity in high-risk paediatric leukaemia PDX cells cultured in vitro, including MLL-rearranged ALL and Philadelphia-positive ALL with IC50 values between 100-400 nM for Auranofin and 30-60 nM for Disulfiram. Induction of apoptosis was evident at 6 hours post Auranofin treatment, or after 12 hours Disulfiram treatment. Each drug significantly increased intracellular ROS as early as one hour post-treatment (p<0.01), which was accompanied by induction of Nrf2, a master regulator of the antioxidant response. Incubation with ROS scavenger N-acetyl cysteine prior to treatment with either drug prevented the increase in cellular ROS levels (p<0.05) and rescued cells from apoptosis (p<0.0001), indicating involvement of reduction-oxidation and increased ROS generation as mechanisms of leukaemia cell death induced by these drugs.

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

P155

TP53 MUTATIONS DISRUPTING DNA BINDING LEAD TO CHEMOTHERAPY RESISTANCE IN ACUTE LYMPHOBластIC LEUKEMIA

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

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

Methods: TP53 was sequenced by the method of Sanger. Drug sensitivity was determined by IC50 in ALL cell lines. Drugs included in the study were DNA damage inducing agents as topoisomerase II inhibitors, alkylating agents, nucleotide analoga, and other agents, most of which are used in ALL relapse treatment protocols.

Results: We identified 20 different TP53 mutations in 34 patients. We classified TP53 mutations into ‘hot spot’ (R175, G245, R248, R273 and R282), non-hot spot and frameshift, respectively. We found that hot spot TP53 mutations were enriched in ALL relapse patients with non-response to treatment compared to good responding patients (64 versus 27%). In ALL cell lines, we colorectal TP53 mutations in Jurkat (R196) and LuCy (V2T2M) and identified R248P in MHH. Three ALL cell lines were TP53 wt (SUP-B15, UOC-B6, NALM-6) and used as controls. Topoisomerase II inhibitors upregulated expression of wt p53. In contrast, nucleotide analoga showed no p53 induc-
tion. IC50 measurements showed that TP53 mutations lead to resistance against common chemotherapeutics 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 relapse patient with non-response to treatment in the MHH 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+wtp53, 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 reduced sensitivity to DNA damage inducing drugs. In contrast to wt p53, R248 did not inhibit cell proliferation under drug treatment. We found that this mutant was unable to induce downstream targets of p53 (p21, BAX).

Therefore, it is our goal to find new T-LBL markers and develop new targeted therapies based on those T-LBL specific markers.

**Aims:**

To explore the oncogenic properties of PIM1 in T-cell acute lymphoblastic leukemia (T-ALL) and T-cell acute lymphoblastic lymphoma (T-LBL) are aggressive immature T-cell malignancies that are considered one disease entity according to the World Health Organization (WHO). Both T-ALL and T-LBL are often characterized by imprinted T-cell receptor (TCR) rearrangements leading to aberrant activation of proto-oncogenes.

**Aims:**

1. To test the hypothesis that PIM1 is an oncogene in T-LBL and suggests that inhibition of this serine/threonine kinase could serve as a novel therapeutic strategy in this aggressive T-cell neoplasm.

**Methods:**

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

- **Aims:**
  - To explore the oncogenic properties of PIM1 in T-cell acute lymphoblastic leukemia (T-ALL) and T-cell acute lymphoblastic lymphoma (T-LBL) are aggressive immature T-cell malignancies that are considered one disease entity according to the World Health Organization (WHO). Both T-ALL and T-LBL are often characterized by imprinted T-cell receptor (TCR) rearrangements leading to aberrant activation of proto-oncogenes.

- **Methods:**
  - We used Targeted Locus Amplification (TLA, de Vree et al., Nat Biotechnol, 2014) to identify a novel translocation (leading to PIM1 kinase overexpression) in a human T-LBL patient. Unraveling the importance of PIM1 activation in these diseases, we first performed RNA sequence and phosphoproteomic studies to identify its downstream targets. T-LBL patient engraftment in NSG mice enabled us to study the therapeutic potential of PIM1 inhibition.

**Results:**

- Applying the TLA technique to identify the location of a novel T-cell LBL driven t(8;7)(p21;p34) translocation in a human T-LBL patient resulting in aberrant activation of the PIM1 proto-oncogene. PIM1 is a constitutively active serine/threonine kinase involved in cell cycle progression, apoptosis, transcription and drug resistance and is overexpressed in a variety of human cancers. Further characterization of this PIM1 rearranged patient sample revealed recurrent genetic alterations that targeted T-LBL/TLB-ONCO and tumor suppressor genes, including NOTCH1, IK2F1, EP300 and CDKN2A. Comparing PIM1 expression between normal T-cell subsets, T-ALL and T-LBL patient samples showed that T-LBL patients express significantly higher PIM1 levels, confirming PIM1 activation is implicated in T-LBL disease biology. Next, we looked at allelic expression ratios of PIM1 and interestingly, we found skewed allelic expression in T-LBL, but not in T-ALL patients. To study the oncogenic properties of PIM1 in the context of malignant T-cell transformation, we did RNA sequencing and phosphoproteomics on the T-ALL/T-LBL tumor line HSB-2 (high PIM1) after PIM1 inhibition with TP5654 (Foucault et al., Neoplasia, 2014). These data revealed that PIM1 inhibition has broad effects on transcription and phosphorylation substrates involved in cell cycle, translation and apoptosis. Finally, we evaluated the therapeutic potential of PIM1 inhibition. Daily TP-3654 treatment (4 weeks) of T-LBL engrafted NSG mice resulted in strong anti-leukemic effects. Currently, we are evaluating if combination of PIM1 inhibition with other immunotherapeutics triggers a more profound anti-leukemic response (Figure 1).

**Summary/Conclusions:**

- All together, our study identifies PIM1 as a putative oncogene in T-LBL and suggests that inhibition of this serine/threonine kinase

![Image](327x345)
Background: B-cell acute lymphoblastic leukemia (B-ALL) is the most common malignancy of childhood and is highly curable with modern risk-adapted chemotherapy. However, 15-20% of children and >60% of adults with B-ALL develop chemoresistance and relapse, indicating need for new therapies. Addition of kinase inhibitors to chemotherapy for patients with BCR-ABL1-rearranged (Ph+) B-ALL has dramatically improved event-free and overall survival, and similar approaches are now under active clinical investigation in patients with BCR-ABL1-like (Philadelphia chromosome-like) or Ph-like B-ALL. Recent studies have demonstrated activated spleen tyrosine kinase (SYK) signaling in various genetic subtypes of B-ALL and preclinical activity of the SYK/FLT3/PUK 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 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

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IKZF1Δ4-7 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. IKZF1Δ4-7, resulting in the dominant negative IK6 isofrom is the most common single IKZF1 deletion.

Aims: We aimed to generate and validate a simple, PCR-based screening assay for IKZF1Δ4-7 using an endpoint PCR assay using primers located in introns 3 and 7. The lower limit of detection was determined by serial dilution of DNA from the IK6-expressing cell line SUP-B15 and calculated to be 0.001%. A total of 95 samples were also tested using the MLPA P335 kit to detect the full spectrum of IKZF1 deletion. Sanger sequencing confirmed the breakpoints in 27 cases.

Results: The median age of the patients tested was 46 years (range 25-65). Overall IKZF1Δ4-7 was detected in 97/490 (20%) patients but the frequency varied by genetic subtype. Patients with BCR-ABL1 fusion had the highest IKZF1Δ4-7 frequency (48/150, 31%) followed by patients with B-other ALL (29/154, 19%). Patients with other classic cytogenetic abnormalities harboured significantly fewer IKZF1Δ4-7 – low hypodiploidy (3/26), MLL gene fusions (3/31), t(1;19) (1/11), high hyperdiploidy (2/9) and iAMP21 (0/3). MLPA did not significantly fewer (29/154, 19%). Patients with other classic cytogenetic abnormalities harboured

Discussion: IKZF1Δ4-7 using an endpoint PCR assay using primers located in introns 3 and 7. The lower limit of detection was determined by serial dilution of DNA from the IK6-expressing cell line SUP-B15 and calculated to be 0.001%. A total of 95 samples were also tested using the MLPA P335 kit to detect the full spectrum of IKZF1 deletion. Sanger sequencing confirmed the breakpoints in 27 cases.

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Table 1.

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

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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. 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 MLL by qualitative detection of different MLL FGTs in infant ALL treated by MLL-Baby protocol.

Methods: Seventy six infants (27 boys and 49 girls) with median age of 5.8 months (range 0.03-11.83) 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-MLLT2-positive, 6 (7.9%) MLL-MLLT10-positive, 4 (5.3%) MLL-EPFS15-positive cases. MRD detection was performed in BM samples by real-time quantitative PCR and nested RT-PCR with sensitivity non-less than 1E-04. MRD-negativity was defined as absence of FGTs in the both assays. Median of follow-up period in the observed group was 6.4 years. Informal consent was obtained in all cases.

Results: We confirmed our earlier finding that the most informative TP for the MLL-Baby study was TP4. MLL-AF4 was detected by FGTs was high-risk arm of MLL-Baby protocol (EFS 0.95±0.09 vs. 0.67±0.05 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 0.90±0.01 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 M1 status of BM on day 15 (HR 3.909, 95% CI 1.456-6.651, p=0.003) and MRD-positivity at TP4 (HR 6.950 95% CI 2.617-18.456) were significant covariates with negative impact on hazard of unfavorable event. Based on dismal outcome of MRL-positive iMR patients we tried to augment their therapy and relocated 5 of them from iMR group to HR group after TP4. Although all patients subsequently relapsed, we also wanted to find out which characteristics might predict relapse in iMR patients who were MRD-negative at TP4 (n=5). Of note, all these 5 relapsed patients (100%) had initial CNS disease while CNS disease was detected only in 2 out of 19 iMR patients (10.5%) who stayed in complete hematological and molecular remission (p=0.003). All 5 relapsed iMR patients who were MRD-negative at TP4 had breakpoint positions within intron 11 of MLL gene and they were MRD-positive by flow cytometry (MRD ≥0.1%) on day 15. None of MRD-negative patients by flow cytometry (MRD <0.1%) on day 15 relapsed later on (p<0.001).

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 in 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.

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Pro-t cell ALL/LLB: an ultra-high risk CD2-negative disease subtype in adults defined by flow cytometry

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

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

Methods: Between 1997 and 2015, 71 adult patients with T-LBL/ALL were treated according to the small ALL05/93 and T-LBL/2004 protocols. Immunophenotype was determined by immunohistochemical staining and by FCM of cellular suspension obtained from lymph nodes (n=31), mediastinal mass (n=12) or nasopharyngeal/perimandibular infiltration (n=2) by fine needle aspiration biopsy (FNAB), as well as of BM (n=10), PB (n=7) and pleural fluid (n=9). Disease subtype was defined according to WHO 2008 classification: pre-T (CD2+/CD3-, cord), cord (CD1a+), medullary/mature (CD3+CD34+). Recognition of pan-T cell CD antigen (pTag) expression included: CD1a, CD2, CD3, CD4, CD5, CD7, CD8. ETP-T-LBL immunophenotype was defined as follows: absent (up to 5% positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD4 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 (LBL): 27%, age<35yrs: 72%, males: 67%, mediastinal mass (MM): 92%, primary CNS+: 8%. Immunophenotype: pro-T: 21%, pre-T: 17%, cort/T pre-T: 25%, medullary/cord: (abstrakt) of pTag present: 0-3: n=25(36%) or 4-7: n=45(64%) of pts. Most frequently expressed pTag were: CD7: 97%, CD5: 87%, CD7: 74%, CD1a: 58%. Myeloid markers: CD13/15 were expressed in 13%/26%/10% and stem cell markers: CD34 in 42% of pts. Overall, 19% (1367) of the study population had ETP-TLLBL/CD1a-CD8-, but CD5 negative: (20-71%), CD45/CD34-CD13/CD15: 100/50% in 50%/50% of pts. 4 pts (31%) with ETP were categorized as pre-T and 9 pts (69%) as pro-T. With a median (95%) follow up of 137 (0.99, 1.733) months, 5-yr OS (95%CI) was 69% (0.547, 0.837), pre-T 48% (0.196, 0.776), mature 40% (0.029, 0.829) and pro-T 10% (0.069, 0.276), respectively. The correlation between EOI and week 10 MRD identified three distinct prognostic groups: both timepoints negative (n=42), RI 14%, RFS 74%; respectively. The correlation between EOI and week 10 MRD identified three distinct prognostic groups: both timepoints negative (n=42), RI 14%, RFS 74%; respectively. The correlation between EOI and week 10 MRD identified three distinct prognostic groups: both timepoints negative (n=42), RI 14%, RFS 74%; respectively.
Background: The outcome for older adults with acute lymphoblastic leukaemia (ALL) is unsatisfactory. The UKALL 12/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 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 (to each treatment is at investigators discretion, including no active therapy). Any elderly patient with newly diagnosed ALL is eligible. There are no exclusions for co-morbidities, including prior malignancies. Baseline characteristics of each group including Charlson index, ECOG, Karnofsky and CRASH scores are being collected. The primary endpoint is the rate of complete remission (CR) after 2 phases of induction. Secondary objectives include determination of MRD status at 3 time points, EFS and OS at 1 year, treatment related mortality and quality of life.

Results: Since December 2012 85 patients have been recruited (4 excluded due to misdiagnosis) with a median age of 67 years (Range 55 – 83). Median follow up is 18.1 months. ECOG performance status was 0 in 33 (41%), 1 in 37 (46%), 2 in 8 (10%) and ≥3 in 3 (4%). Treatment allocation has been Ph+ n=18, Intensive n=34, non-Intensive n=11, Intensive + n=7, and Registration only n=11 patients. It is too early to perform a full analysis of the reasons given for choosing each regimen but age appears to be a major factor for Ph-ve patients, with a median age of 74 years (Range 64-82) in the non-Intensive arm compared with 66 years (Range 56 -76) in the Intensive and Intensive+ arms. A total of 36/61 (57%) patients had high risk cytogenetics including BCR-ABL1 (n=21), low hypodiploidy (n=10), complex karyotype (n=1) and KMT2A- AFF1 (aka MLL-AF4) (n=4). Charlson index and CRASH score data is awaited. At the end of 2 phases of treatment on Arm A (Ph+ve) 17/18 (94%) patients achieved CR. On Arms B-D 27/52 (52%) patients achieved CR. Grade 3/4 AEs were seen in the majority of patients. The most common toxicities were haematological and infections. So far 30 relapses have been reported, 25 are isolated mediulary relapses, 4 isolated CNS and combined in 1 patient. To date, 41 deaths have been reported; 32 patients died of ALL, 7 of infection, 1 cardiac and 1 multi-organ failure. Fifty one patients have had a PFS event. The median PFS is 13.2 months in Arm A (Philadelphia +ve) and 11.3 months Arm B-D. The median OS is 19.5 months in Arm A (Philadelphia +ve) and 15.5 months in Arms B-D (Figure 1).

Predictors of survival

Patient characteristics, chemotherapeutic regimens and baseline characteristics are collected. Baseline characteristics of each group including Charlson index, ECOG, Karnofsky and CRASH scores are being collected. A total of 36/61 (57%) patients had high risk cytogenetics including BCR-ABL1 (n=21), low hypodiploidy (n=10), complex karyotype (n=1) and KMT2A- AFF1 (aka MLL-AF4) (n=4). Charlson index and CRASH score data is awaited. At the end of 2 phases of treatment on Arm A (Ph+ve) 17/18 (94%) patients achieved CR. On Arms B-D 27/52 (52%) patients achieved CR. Grade 3/4 AEs were seen in the majority of patients. The most common toxicities were haematological and infections. So far 30 relapses have been reported, 25 are isolated mediulary relapses, 4 isolated CNS and combined in 1 patient. To date, 41 deaths have been reported; 32 patients died of ALL, 7 of infection, 1 cardiac and 1 multi-organ failure. Fifty one patients have had a PFS event. The median PFS is 13.2 months in Arm A (Philadelphia +ve) and 11.3 months Arm B-D. The median OS is 19.5 months in Arm A (Philadelphia +ve) and 15.5 months in Arms B-D (Figure 1).

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 LYMPHOBLASTIC 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 should 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) age ≥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 (0.23-126), during which time 49 (63%) deaths occurred; 25 (35%) related to the disease, & 15 (23%) 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 6.2 x 10⁹/l(0.5-160.8), 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%) splenomegaly, 6/60 (10%) 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 (25 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, 3 (6%) 11q22.3(4;11) MLL-AF4, 2 (4%) t(1;19) E2A-PBX1, 1 (2%) KIF1F deletion. Treatment and Outcomes: 10 (16%) pts received palliative therapy only, which included TKIs, chemotherapy, or hospice. The other 53 (84%) received induction chemotherapy. Only 12 (23%) had an up-front dose reduction due to comorbidities. 32 (60%) received Hyper-CVD, concomitantly with rituximab in 11 (34%) pts. & TKIs in 9 (28%) pts. 21 (40%) pts received other regimens, of which 14 (67%) had asparaginase-based chemotherapy. Only 2 (4%) pts who received induction chemotherapy died within the first 60 days; both received Hyper-CVD. Median number of cycles to achieve CR was 1 (1-8) with CR/CRi rate of 93%, & median time to CR1 was 34 days (19-459). 3 pts who underwent palliative chemotherapy achieved CR (all had Ph+ disease & received TKIs). 7 pts (13%) had primary induction failure. 50% of pts relapsed within a median time of 12.6 (3.6-72.8) months. Only 10 pts under- went allogeneic hematopoietic stem cell transplantation (HSCT), of which 2 (20%) relapsed in less than 180 days. Median survival after HSCT was not reached. Predictors of survival: Elderly ALL has worse mOS compared to our adult ALL cohort, 17.2 (IQR; 11.7-32.9) vs 52.1 (IQR; 27.6-169.9) mon (p=0.0016). In a univariate analysis model which included multiple variables, only ECOG PS ≥2, WBC>30,000, CDKN2A del, & CNS leukemia were statistically significant, however only CNS leukemia (p=0.0009) & WBC (p=0.0168) retained statistical significance in multivariate mode, with a trend in CDKN2A del (p=0.06) (Figure 1).

Figure 1.

Summary/Conclusions: Elderly pts with ALL have worse survival compared to younger adults. However, this was not reflected by a low CR rate, or a high rate of mortality during induction, but by grim disease overall. We report for the first time the incidence of 20% for CDKN2A del in this disease group. Further studies are needed to confirm this finding, as it could be a target for novel therapies.

P166

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

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Background: Elderly acute lymphoblastic leukaemia/lymphoma (ALL) is a rare
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). Refractoriness was more frequent in p210+ than in p190+ cases (12% vs 5%): this finding did not translate into significantly different OS and DFS (30% vs 48% and 32% vs 51%, respectively). When patients were stratified by age, adults had a significantly better OS and DFS at 100 months than elderly (53% vs 19%, p= 0.04, and 57% vs 20%, p= 0.03, respectively), even more marked in multivariate analysis treated with TKI alone (75% vs 20%, p=0.01 and 73% vs 21.4%, p=0.017, respectively). The TKI used (imatinib or dasatinib) did not impact in adults, while a significant advantage in OS and DFS was observed for elderly patients receiving dasatinib (Figure 1): this might be due to the greater activity of dasatinib and also highlights the importance of consolidation chemotherapy, performed almost exclusively in adults. Considering adults only, within the chemotherapy+TKI group, 5 patients were transplanted and 19 were not: all transplanted cases are in CR, while in the non-transplanted cases 6 are in CR, 11 have relapsed and 2 have died in CR (p=0.01); within the TKI group, 8 patients underwent a SCT and 15 died out of the transplanted cases, 6 are in CR and 2 have died due to complications, while 11 of the non-transplanted patients are in first CR, 3 have relapsed and 1 has died in first CR (p=n.s.). Of the 5 patients transplanted in second CR, only 1 is alive.

Figure 1.

Summary/Conclusions: This study further underlines the benefit of an induction based on a TKI alone. Since age holds strong prognostic significance, our results suggest that while TKI followed by consolidation chemotherapy is the optimal choice for adults, in elderly cases dasatinib is more appropriate, since patients are often unfit to receive further chemotherapy. Finally, the advantage of SCT needs to be carefully redefined in the TKI era.
Results: Our series included 69 boys and 57 girls diagnosed with acute leukemia, with a median age of 6.1 years (range 0-17.4 years). We included 12 infant patients (<1 year old). Eighty-two (65%) patients had B-cell precursor acute lymphoblastic leukemia (BCP-ALL), 24 patients T-LL and 20 patients had acute myeloblastic leukemia (AML). Globally, we found higher expression levels of class I HDAC isoforms (HDAC 1, 2, 3 & 8) in leuemic samples as compared to non-leuemic samples, as previously reported. Interestingly, some HDAC isoforms associated with specific genetic aberrations. Those patients with rearrangement of MLL (KMT2A) gene (n=18, including 9 BCP-ALL and 9 AML; 7 infants and 11 pediatric) showed a significantly higher expression of HDAC9 (p=0.0001) and a statistically significant underexpression of HDAC1 and HDAC3 (p=0.003 and 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 63%. The presence of MDD was associated with a worse OS compared to patients without MDD (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 (69% 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).

Figure 1.

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.

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MINIMAL DISSEMINATED DISEASE DETECTION BY FLOWCYTOMETRIC IMMUNOPHENOTYPING IN T-CELL ACUTE LYMPHOBLASTIC LYMPHOMA
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Background: T-cell acute lymphoblastic lymphoma (T-LBL) with minimal disseminated disease (MDD) is defined as the presence of T-LBL with <25% blasts in the peripheral blood (PB) and/or bone marrow (BM) by morphology and the presence of immunophenotypically abnormal T-lymphoblasts in bone marrow by flowcytometry. Published literature regarding the prevalence and clinical significance of this rare subgroup is sparse. In this study we analysed the prevalence of minimal disseminated disease in T-LBL with <25% blasts in PB and BM using 8-10 colour flowcytometric immunophenotyping and evaluate the clinical and immunophenotypic features.

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

Results: A retrospective analysis of 42 cases of T-LBL with <25% blasts in peripheral and bone marrow was done. The mean age was 12.2 years (Range:2-48 years). M:F ratio was 1:1.7. Nearly all patients had normal haemoglobin, total leukocyte count and platelet counts. LDH was raised in majority of the patients (Mean 647U/l; N=1800U/l). CSF examination was negative in all cases indicating that it is unlikely to have CNS involvement in patients with <25% blasts in PB and BM. Minimal disseminated disease was seen in 12 cases (12/42=28.6%) of cases. Of the 12 cases with minimal disseminated disease two cases were 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 in BM. 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.

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.

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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 RANDOMIZED TRIAL
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Background: Older patients (pts) with acute lymphoblastic leukemia (ALL) have poor tolerance of intensive chemotherapy, and novel strategies are needed in this population. In pts with relapsed/refractory ALL, inotuzumab ozogamicin (InO), an anti-CD22 antibody-drug conjugate, has been shown to improve survival compared to salvage chemotherapy.

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

Methods: Pts ≥60 years of age with newly diagnosed Philadelphia chromosome-negative pre-B received mini-Hyper-CVD (compared to hyper-CVAD: no anthracycline, 50% dose reductions of cyclophosphamide and dexamethasone, 75% dose reduction of methotrexate, 85% dose reduction of cytarabine). InO was given on day 3 of the first 4 cycles. The first 6 pts received InO at a dose of 1.3 mg/m2 for cycle 1 followed by 0.8 mg/m2 for cycles 2-4. 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 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. In pts after 8 cycles then received POMP maintenance for up to 3 years.
Results: Between 4/2012 and 12/2016, 47 pts have been treated, 4 of whom had received 1 cycle of prior therapy and were in CR at the time of enrollment. The median age was 68 years (range, 60-81), and median CD22 expression was 97% (range, 72-100%). Of 43 pts evaluable for response, 42 responded (ORR=98%). Best response was CR in 36 pts (84%), CRp in 5 (12%) and CRi in 1 (2%). MRD negativity by 6-color multiparameter flow cytometry was achieved in 31 of 41 evaluable pts (76%) on day 21 and in 44 of 46 evaluable pts (96%) within 12 weeks of treatment. The median follow-up was 24 months (range, 15-55 months), 3 pts (6%) underwent allogeneic stem cell transplantation (ASCT) in first remission. Of the 46 responders, 6 pts (13%) have relapsed. 16 pts have died, 1 due to resistant disease, 4 after relapse, 1 after ASCT and 10 in CR/CRp. 21 pts remain on treatment (consolidation, n=3; POMP maintenance, n=19), and 5 pts have completed all therapy. The 3-year continuous CR and OS rates were 72% and 54%, respectively. Compared to a historical cohort of 79 older pts treated at our institution with hyper-CVAD ± rituximab, mini-hyper-CVAD+InO resulted in significantly improved OS (3-year OS rate: 54% vs 31%; median OS not reached versus 16 months; P=0.007).

Summary/Conclusions: The combination of InO with mini-hyper-CVAD 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.
Background: The branched chain amino acids (BCAAs) valine, leucine, and isoleucine are essential AAs for the human body. The activity of BCAA metabolism, especially the export of the enzyme BCAA Transaminase 1 (BCAT1) has recently been associated with aggressiveness in several cancer entities. However, the mechanistic role of BCAT1 in this process remains uncertain.

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

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

Results: We performed high-resolution proteomic analysis of human acute myeloid leukemia (AML) stem cell (LSC) and non-LSC populations, which have been functionally validated by xenotransplantation into NSG mice, and we found the BCAA pathway enriched and BCAT1 overexpressed in LSCs. We show that BCAT1, which transfers α-amino groups from BCAAs to α-ketoglutarate (αKG), is a crucial regulator of αKG homeostasis and plays a central role in the regulation of the branched chain amino acid (BCAA) cycle. BCAT1 overexpression leads to increased αKG levels, which in turn activates the mammalian target of rapamycin (mTOR) pathway, leading to enhanced cell proliferation and survival. We further demonstrated that BCAT1 knockdown results in decreased proliferation and survival of AML cells, suggesting that BCAT1 is a therapeutic target to compromise LSC function in AML patients.

Conclusion: Our findings provide a novel mechanistic link between BCAA metabolism and AML stem cell biology, and identify BCAT1 as an important therapeutic target for AML treatment.

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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 lncRNA HOXB-AS3, which is embedded in the HOXB locus, has been previously identified among the lncRNAs that associated with mutated NPM1 (NPM1mut) in CN-AML.

Aims: Our aims were to evaluate the biologic significance of HOXB-AS3 expression in NPM1mut.AML patients.

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 Basecapture probes (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 identified a previously unannotated (NR_033203/ENST0000491264) and 1 novel variant of HOXB-AS3. NPM1mut pt samples exhibited higher expression of HOXB-AS3 compared to those with wild-type (WT) NPM1 (P = .001) and healthy donors (P = .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 = .02). HOXB-AS3 KD also led to a reduction in the number of formed colonies by OCI-AML3 cells in colony-forming assays (P = .002). HOXB-AS3 KD in NPM1mut pt blasts (n = 5) led to a decrease in the number of formed colonies (P = .001). To evaluate the effect of HOXB-AS3 KD in vivo we generated aحادث 1 pt-derived xenograft (PDX) model by engrafting NSG mice with blasts of a NPM1mut pt. Treatment of the engrafted mice with nanoparticle-formulated anti-HOXB-AS3 gapmers led to significant prolongation of survival compared to treatment with non-targeting control gapmers in 2 independent experiments (P = .01 and P = .005). Mass spectrometry and comparative proteomic analysis of HOXB-AS3 and U1-specific RNA-protein complexes identified EB1 and NPM1 as candidate HOXB-AS3-binding proteins. RNA-immunoprecipitation experiments validated the interaction of HOXB-AS3 with EB1 (20-fold increase of HOXB-AS3 abundance in EB1-precipitate compared to normal IgG control, P = .001). Direct visualization of HOXB-AS3 showed co-localization of the lncRNA and WT NPM1 in the nuclei of OCI-AML3 cells. HOXB-AS3 has been previously shown to interact with NPM1 and to regulate ribosomal biogenesis and growth of AML cells (Nguyen et al., 2016). We hypothesized that HOXB-AS3 could affect the EB1-NPM1 interaction and impact on the ribosomal biogenesis process. In consistency with this hypothesis, HOXB-AS3 KD led to a decrease in the transcription of rRNA species in OCI-AML3 cells (P = .001) and in vitro-treated blasts of 2 NPM1mut pts (P = .001). HOXB-AS3 KD also led to a reduction of protein synthesis in the AML cells, as measured by incorporation of fluorochrome-tagged tracers in newly translated polypeptides.
Summary/Conclusions: Conclusions: HOXB-AS3 is strongly associated with NPM1 mutations in AML. HOXB-AS3 interacts with ESB1 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.

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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. Souers et al Nat Med 2013 and Roberts et al., NEJM 2016). BCL-XI (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 MCL1) were obtained from patients providing informed consent. For in vivo experiments, NSG; NOD.Cg-Prkdcscid Il2rgtm1Wjl/SJ (NSG) or NOD/Rag -/-/ (NRGS) 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 MLLT3A. Patient-derived xenografts treated with rapid reduction of established AML in the bone marrow one week of treatment with S55746 and S63845 (Figure 1D). Safety and tolerability of this approach was confirmed using normal CD34+ stem and progenitor cells in short-term cell culture (48h) and long-term (2-3 weeks) clonogenic assays and from histological and biochemical examination of mice receiving treated for up to 8 weeks at doses shown to be highly efficacious against AML.

Summary/Conclusions: Dual BH3-mimetic targeting of BCL-2 and MCL1 induces rapid and synergistic cytoreduction of human AML cell line and primary AML samples in vitro and in vivo across a diverse range of AML genotypes. We therefore support for the in vivo use of the dual pharmacological agents, both in AML cell lines and MCL-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.

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

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

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

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

Results: While no leukemias or tumors developed in PmlC62A/C65A mice, the forced dimerization of RARα - mediated artificially by linking RARα to the dimerisation domain of the Nfkb 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 improved the survival of mice transplanted with PML-RARA leukemic blasts, but not with PmlC62A/C65A-p50-RARα, revealing the essential role of NBs for an effective response to differentiating drug. While formation of the PML-RARA fusion is considered an 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-RARα leukemia as compared to PmlWT-P50-RARα, possibly due to the inability to properly recruit DNA repair players (Figure 1). Using DNA repair reporter assays, we demonstrated that DNA repair via both non-homologous end joining (NHEJ) 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-RARα 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 analyzing 53BP1 and γH2AX foci formation, and clearance, was not affected. None of the DNA repair player 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 (p<0.04; quantified using Amnis ImageStreamX Mk II imaging flow cytometer). Additionally, we found that Rad51 foci showed a non-linear correlation post-IR, and γH2AX was expressed, with impairment of Rad51 co-localisation and interaction with γH2AX.}

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

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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/-7q) 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 neoplasms are elicited by accumulation of cooperating mutations and a more thorough study of isolated mutations need to be undertaken until all leukemic processes guiding transformation, 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 quantifiable genetic cooperation screenings was established. Four out of six CRISPR-Cas9 pools tested – comprised of five genes each and allowing fast and quantifiable genetic cooperation screenings was established. We thus tested every oncogene/tumor suppressor pool from the in vitro setting in a murine bone marrow transplantation model with newly transduced LSK cells which resulted in robust induction of leukemia. Analysing the in vitro immortalized clones yielded robust engraftment and then 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 newly transduced LSK cells which resulted in robust induction of leukemia. Analysing the in vitro immortalized clones yielded robust engraftment and then lineage contributions in mice but no overt leukemia was detected. We thus tested every oncogene/tumor suppressor pool from the in vitro setting in a murine bone marrow transplantation model with newly transduced LSK cells which resulted in robust induction of leukemia. Analysing the in vitro immortalized clones yielded robust engraftment and then lineage contributions in mice but no overt leukemia was detected.

Summary/Conclusions: Our study highlights the power of the CRISPR-Cas9 system to probe oncogenic interaction. Mutational CRISPR screenings in vitro, and a newly established in vitro CRISPR-Cas9 immortalization assay for high throughput screening of sgRNA pools, delivered potential cooperating partners of EzH2 loss in AML, and provides rich cellular resources to identify molecular mechanisms of oncogenic synergies and dependencies.

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Abstract withdrawn.

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ACUTE MYELOID LEUKEMIA EVOLUTION CAN BE RECONSTRUCTED BY ANALYSIS OF NON-LEUKEMIC CELLULAR SUBCOMPARTMENTS AND MULTILINEAGE ENGRAFTED MICE
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Background: The Enhancers of Polycomb (EPC) proteins 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 complexes 2 (PRC2). NuA4 and PRC2 are two major chromatin modifying complexes encompassing opposing epigenetic activities and both are known to be deregulated in AML. A systems biology approach to understand the 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 lines, and Chromatin immunoprecipitation (ChIP) was performed using High cell ChIP Kit and iPure kit V2 (Diagenode) followed by NextSeq500 Illuma sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChippeakAnno. Lentiviral supernatants were prepared and THP1 cells were infected with viral particles containing pLKO1 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, Smc3, Stag2 in AML cell lines, and Chromatin immunoprecipitation (ChIP) was performed using High cell ChIP Kit and iPure kit V2 (Diagenode) followed by NextSeq500 Illuma sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChippeakAnno. Lentiviral supernatants were prepared and THP1 cells were infected with viral particles containing pLKO1 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, Smc3, Stag2 in AML cell lines, 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.

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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 two major chromatin modifying complexes encompassing opposing epigenetic activities and both are known to be deregulated in AML. A systems biology approach to understand the essential role of the Enhancers of Polycomb (EPC) proteins EPC1 and EPC2 in AML.

Aims: To define 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 < 0.20, Somatic Intervals > FRQ > FDR > 99%, SNV 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 wild 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 cases with a single mutated gene (i.e. Trisomy 8, or STAG2, NRAS and KIT). Tracking of AML-specific mutations in non-leukemic T- and B-cells showed that some AML mutations like DNMT3A, IDH1, IDH2, EZH2 and ZNF536 were already detectable in T- and B-cells indicating their pre-leukemic status. Furthermore, analysis of multi-lineage engrafted xenografts detected leukemia-specific mutations in human myeloid and lymphoid sub-compartments suggesting that these animals were engrafted from functionally normal pre-leukemic HSC. To reconstruct the sequence of pre-leukemic mutations single-cell HSC were seeded and the resulting colonies analyzed for the presence of the respective leukemia specific mutations. Based on the different mutational data, combined with the cellular context in which these were detectable the leukemic evolution of most patients could be reconstructed. In one patient we detected a DNMT3A mutation in myeloid and lymphoid cells, whereas NPM1 and FLT3-ITD mutations were only detectable in leukemic cells proving the pre-leukemic status of DNMT3A in this case. In another patient we found DNMT3A and IDH2 in T- cells whereas Trisomy 8 and a STAG2 deletion were only detectable in leukemic cells. By analyzing colonies from single cell HSC we were able to detect complex pre-leukemic hierarchies with one example in which a ZNF536 mutation could be identified as initiating event that hasn’t been described in leukemia yet.

Summary/Conclusions: WES 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 monocytic differentiation inducer MAFB, the H2A ubiquitin ligase TRIM37 and the pro-apoptotic tumor suppressor CMTM3.

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

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STROMA-DERIVED FACTORS STIMULATE JAK/STAT SIGNALING IN AML CELLS RESULTING IN RESISTANCE TO BCL2 INHIBITOR VENETOCLAX

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Background: The bone marrow (BM) microenvironment is known to protect AML cells from drug therapy. We showed earlier that conditioned medium (CM) from the BM stromal cell line HS-5 increased cell viability and led to resistance to specific drug classes.

Aims: Here, we investigate the mechanisms binding the BM stromal cell induced resistance to venetoclax and its reversal by ruxolitinib.

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

Results: To identify the factors contributing to BM mediated drug resistance of AML cells, we analyzed the effect of IL-6, IL-8, MIP-3α, GM-CSF and G-CSF, cytokines enriched in the HS-5 CM, on proliferation of MNCs collected from AML patients. GM-CSF and to some extent G-CSF alone confer resistance to venetoclax similar to CM that we showed earlier to reduce sensitivity to BCL2 inhibitors. To identify the impact of stroma-derived factors on cellular signaling we stimulated AML patient cells with CM and analyzed the phosphorylation of STAT3, STAT5, ERK and AKT. Compared to control conditions, CM rapidly induced phosphorylation of STAT3 in primary AML cells. When the effect of individual cytokines was tested, we noted that GM-CSF and G-CSF alone could mimic the effect of CM on cellular signaling. Gene expression data showed the receptor for GM-CSF (CSF2RA) is more highly expressed in AML patient cells compared to healthy controls. Taken together, these results show that cytokines such as GM-CSF from BM stromal cells increase JAK/STAT signaling, which may lead to enhanced survival of AML cells. To determine whether the protective effect of stroma on BCL2 inhibition was dependent on cell-to-cell interactions we cultured AML patient cells either in direct contact with MSCs or separated from stroma with a 0.4 μm pore membrane. 48h treatment with 100 nM venetoclax did not result in significant reduction of CD34+ AML cells regardless of whether AML cells were directly cultured with stroma or separated by a membrane, further indicating that stroma-derived soluble factors are sufficient to reduce sensitivity to venetoclax. Since the most abundant cytokines secreted by HS-5 cells, GM-CSF and G-CSF led to increased phosphorylation of STAT5 in primary AML cells, we tested the combination of venetoclax and JAK1/2 inhibitor ruxolitinib. We found that ruxolitinib potentiated sensitivity to venetoclax when tested with AML patient cells in HS-5 CM and in co-culture and transwell assays. Significantly, the combination was more effective at reducing tumor burden in a xenograft mouse model of AML than either drug alone.

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

**Aims:** We have hypothesized that cytogenetic aberrations in CK-AML create genetic perturbations that recur across patients, non-tumorigenic 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 multiple independent algorithms (as paired reads that flank, or single-reads that span the junction). We also sequenced frequently mutated genes in AML (e.g., CEBPα, MYB) and downstream STAT5 (pSTAT5) were evaluated by immunoblotting. Phospho-STAT5 (pSTAT5) was measured by immunoblotting. Phospho-STAT5 (pSTAT5) was measured.

**Results:** We identified 54 gene fusion events in 30 of the 65 cases (46%) with up to four fusions per case. All fusions are supported by array-based genomic profiling and/or long range PCR, respectively. About 35% of the fusions were in-frame, encoding chimeric proteins. The remainder encoded either C-terminally truncated 5' fusion partners or N-terminally truncated (or rarely full-length) 3' fusion partners, which contributed only the 5'UTR. In many instances, the fusions are predicted to lead to the overexpression or chimeric activation of known or putative novel cancer genes. Of the 54 fusions, only three (RUNX1-MECOM, MN1-ETV6, and ETV6-MEOM1) were previously reported in AML. The most frequently affected genes were RUNX1 (n=5), KMT2A, and MLL (n=3 each). Identified gene fusions included in part independently validated by array-based genomic profiling and/or long range PCR, respectively. About 35% of the fusions were in-frame, encoding chimeric proteins. The remainder encoded either C-terminally truncated 5' fusion partners or N-terminally truncated (or rarely full-length) 3' fusion partners, which contributed only the 5'UTR. In many instances, the fusions are predicted to lead to the overexpression or chimeric activation of known or putative novel cancer genes. Of the 54 fusions, only three (RUNX1-MECOM, MN1-ETV6, and ETV6-MEOM1) were previously reported in AML. The most frequently affected genes were RUNX1 (n=5), KMT2A, and MLL (n=3 each). Identified gene fusions included in part independently validated by array-based genomic profiling and/or long range PCR, respectively. About 35% of the fusions were in-frame, encoding chimeric proteins. The remainder encoded either C-terminally truncated 5' fusion partners or N-terminally truncated (or rarely full-length) 3' fusion partners, which contributed only the 5'UTR.

**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 partner, 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.

**Figure 1.**

**Summary/Conclusions:** the biomarker H3K27me3 HIST1high is correlated with a better OS and LFS in NPM1mut CN-AML patient, independently of other known genetic alterations in particular FLT3/ITD. The worse outcome of FLT3-wt H3K27me3 HIST1low patients is concomitant with high expression of replication-dependent histone genes that could explain treatment failure.
tivity 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% pFL3T 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 pFL3T and pSTAT5, including 4 mutations with >50% pFL3T. All mutations that demonstrated aberrant pFL3T also had aberrant pSTAT5; however, a direct correlation of pFL3T 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 growth hyperproliferation demonstrated that in every case of aberrant activation, crenolanib resulted in potent inhibition of phosphorylation of FLT3 and STAT5 with an IC50 range of 1.3-13.9 nM and 0.6-6.5 nM respectively. Many of the mutations tested were exquisitely sensitive to crenolanib, with 9 of 10 mutations tested demonstrating an IC50 of pFL3T inhibition ≤0.6 nM. Inhibition of downstream kinases is necessary for optimal efficacy of any FLT3 inhibitor and phosphorylation of STAT5 was potently inhibited by crenolanib in all cases. Quizartinib inhibited pFL3T and pSTAT5 with an IC50 range of 1.8-15.11 nM and 1-33.9 nM respectively, demonstrating less effective inhibition specifically at mutations including D835Y, D839E, N676K, 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.

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Abstract withdrawn.

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THE BCL-2 INHIBITOR VENETOCLAX INHIBITS NRF2 ANTIOXIDANT PATHWAY ACTIVATION INDUCED BY HYPMETHYLATING AGENTS IN ACUTE MYELOID LEUKEMIA

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Background: The selective Bcl-2 inhibitor Venetoclax (ABT-199) has shown potential in clinical trials against Acute Myeloid Leukemia (AML) in preclinical and early clinical studies and impressive results have been achieved using the combination of hypomethylating agents (HMA) with venetoclax suggesting synergy between these agents.

Induction of Reactive Oxygen Species (ROS) is important for the cytotoxicity of various AML therapies including HMA. Induction of ROS by various cytotoxic therapies concurrently activates the Nrf2 antioxidant response pathway which in turn results in induction of antioxidant enzymes that neutralize ROS. Upon ROS induction, the transcription factor Nrf2 is released from its adaptor protein Keap 1 in the cytoplasm whereby Nrf2 enters the nucleus and binds to antioxidant response element sequences in the promoters of various genes. Nrf2 pathway activation has been shown to mediate chemoresistance in various cancers including AML. Low ROS levels have been shown to be a hallmark of leukemia stem cells and are critical to their self renewal capacity. In this study, we examined whether Nrf2 inhibition is an additional mechanism responsible for the antileukemic activity of 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 of 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 and Keap 1 complex was assessed by Western blot analysis, immuno precipitation 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 resulted in increased nuclear translocation of Nrf2 (Figure 1) and induction of downstream antioxidant enzymes including HO-1 and NQO1. Immunofluorescence studies confirmed the induction of nuclear translocation of Nrfl2 by venetoclax. Immunoprecipitation studies indicated that Bcl-2, Keap 1 and Nrf2 associate in a protein complex in the cytoplasm and that treatment with venetoclax leads to dissociation of Bcl-2 from the Nrf2/Keap 1 complex and targets Nrf2 to ubiquitination and proteasomal degradation.

Figure 1.

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

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UNRAVELING EPIGENOMIC REGULATION IN THE EVOLUTION OF RELAPSING PEDIATRIC AML

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

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

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

Results: All genomic regions that were significantly enriched by H3K27ac were analyzed, resulting in ~30.000 active promoters and enhancers per sample. Genome-wide Pearson correlation of all enriched regions showed subclustering of patients based on molecular aberration. Interestingly, epigenomic analysis showed that the initial diagnosis (Dx) and the patient’s relapse (Rel) sample were highly correlated. Also, single-cell RNA-seq analysis identified two highly identical homogeneous populations at Dx and Rel. Following the fact that no major differences were observed between AML cells at diagnosis and relapse, NRPs were analyzed. Here striking differences in H3K27ac enrichment were observed in MLL-rearranged patients between NRPs and RPs. Enhancers and promoters were differentially enriched at diagnosis, of which Sphk1, a kinase involved in proliferation and survival, was significantly more enriched in RPs, while the promoter of transcription factor ELF1 was nearby located enhancers were active in NRPs only.
Background: The complex pathogenesis of cancer often necessitates combination therapies to optimize patient benefit. Thus, we investigated preclinical combinations of SY-1425 (tamborinase) and other agents to build on the monotherapy strategy with SY-1425 in biomarker selected AML and MDS patients (Phase 2 study, NCT02807558). Based on the RARα mediated myeloid gene activation of SY-1425, epigenetic priming with hypomethylating agents (HMAs) and CD38 induction were explored.

Aims: We sought to investigate mechanistically informed combinations of SY-1425 with known myeloid agents and with novel agents in AML. We hypothesized that the HMA azacitidine could prime AML cells for SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA).

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

Results: RARα acts as a repressive transcription factor until bound by SY-1425 leading to potent, targeted activation of myeloid genes. HMAs can further prime this activation by depleting repressive methylation of these target genes. The combination of SY-1425 and azacitidine showed synergy in RARA-high AML cell lines, but not in RARA-low AML cell lines, with combination indices less than 0.5. Co-administration in a RARA-high PDX model demonstrated superior reduction of tumor burden (<1% detectable tumor cells) vs either treatment alone (64% decline in SY-1425 and 8% with azacitidine). Various combination regimens evaluated in the PDX model over two cycles (56 days) found that 1 week of azacitidine followed by 3 weeks of SY-1425 maximized for anti-tumor activity (<5% AML cells in periphery, bone marrow and spleen) and tolerability (<8% weight loss). RARα binds directly to the CD38 locus and induces H3K27 acetylation upregulating CD38 expression of the myeloma cell surface CD38 mRNA transcripts in RARA-high models. SY-1425 treatment of four RARA-high AML cell lines and three RARA-high primary AML patient samples induced cell surface CD38 to high levels comparable to those of DARA sensitive multiple myeloma cells. In contrast, no CD38 induction was observed in RARA-low cell lines. RARA-high AML cell lines treated with SY-1425 and DARA were six fold more sensitive to NK cell mediated ADCC compared to single agent treatments and exhibited a 5-10 fold increase in NK cell-dependent activation measured by IFNγ secretion.

Summary/Conclusions: The RARα biomarker dependent synergy with azacitidine and SY-1425 is hypothesized to work through hypomethylation based priming of myeloid differntiation by SY-1425 agonism of formerly repressed RARα target genes. Since CD38 is one of the most strongly induced RARα target genes in response to SY-1425, AML blasts can be sensitized to DARA in a biomarker dependent manner. The preclinical synergistic effects and anticipated non-overlapping clinical toxicity profiles of the respective agents provide a strong rationale for clinical evaluation of each SY-1425 combination in biomarker selected AML and MDS patients.
decreased expression of IGFBP7 might be associated with decreased chemothera-
paly 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 overex-
pressed 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 sur-
vival advantage of IGFBP7−AML cells during chemotherapy treatment. Importantly,
increasing 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 IGFB-
7 to the currently used chemotherapy regimens might be a promising strat-
egy to specifically eradicate LSCs and decrease AML relapse rates.
indeterminate potential (CHIP), DNA methylation, and splicing pathways had low rate of MC, whereas mutations in transcription factors or receptor tyrosine kinase (RTK) had high rate of MC. Pts who achieved MC1.0 (median 31.2 vs 12.5 months, P<0.04) or CMC (median 31.2 vs 12.5 months, P=0.049) had significantly better relapse-free survival (RFS).

Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>MCL2 (%)</th>
<th>MCL1 (%)</th>
<th>CMC (%)</th>
<th>Pathway</th>
<th>MCL2 (%)</th>
<th>MCL1 (%)</th>
<th>CMC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUNX1</td>
<td>21%</td>
<td>17%</td>
<td>14%</td>
<td>CHIP-associated</td>
<td>33%</td>
<td>24%</td>
<td>22%</td>
</tr>
<tr>
<td>TFET</td>
<td>100%</td>
<td>99%</td>
<td>99%</td>
<td>TFET-associated</td>
<td>39%</td>
<td>39%</td>
<td>39%</td>
</tr>
<tr>
<td>TFET</td>
<td>15%</td>
<td>15%</td>
<td>15%</td>
<td>RTK pathway</td>
<td>88%</td>
<td>87%</td>
<td>86%</td>
</tr>
<tr>
<td>TFET</td>
<td>15%</td>
<td>15%</td>
<td>15%</td>
<td>TFET-associated</td>
<td>89%</td>
<td>87%</td>
<td>83%</td>
</tr>
<tr>
<td>CEBPA</td>
<td>100%</td>
<td>89%</td>
<td>89%</td>
<td>Chromatin-Coher</td>
<td>67%</td>
<td>57%</td>
<td>53%</td>
</tr>
<tr>
<td>ID2</td>
<td>38%</td>
<td>44%</td>
<td>38%</td>
<td>Splicing</td>
<td>35%</td>
<td>17%</td>
<td>17%</td>
</tr>
</tbody>
</table>

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

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

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

Methods: We conducted a nationwide population-based cohort study and included AML patients ≥25 years diagnosed in Denmark 2000-2014 (end of follow-up, Feb 2016). KM curves and Cox regression (Hazard ratios; HRs) was included AML patients ≥25 years diagnosed in Denmark 2000-2014 (end of follow-up, Feb 2016). KM curves and Cox regression (Hazard ratios; HRs) was included AML patients ≥25 years diagnosed in Denmark 2000-2014 (end of follow-up, Feb 2016).

Results: Of 2992 patients, 1588 (53.1%) received remission induction (odds ratios; ORs) to compare treatment intensity, chance of clinical trial inclusion, and complete remission (CR) between groups. Results were given (CI=0.61-0.99), medium 0.99 (CI=0.84-1.16), and low 1.03 (CI=0.84-1.27).

Summary/Conclusions: In Denmark where health-care is free and uniform, high SE status does not affect treatment intensity in younger patients or response to therapy. However, educational level, but not income, influences all-risk CR 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.

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

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 ≥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. Only one RUNX1 mutation was identified. Significantly, the germline variants occurred mutually exclusive of the somatic variants. Out of 20 patients with germline RUNX1 mutations, 16 had co-occurring known pathogenic mutations in AML-related genes. Most significantly, 62% (10/16) and 51% (14/27) of patients with germline or somatic RUNX1 mutations, respectively, had ≥2 co-occurring AML-related pathogenic mutations that were exclusive to their cohort (Table 1). Both germline and somatic RUNX1 mutational cohorts had ≥12 overlapping co-occurring mutations. The most common mutations, for both groups, were in FLT3 (4/143), ASXL1 (8/43), and IDH2 (7/43) (Table 1). Patient demographics and treatment-related outcomes were similar for both cohorts.
Methods: multiple LSC markers on the outcome of AML patients. Eligible patients were enrolled from September 2010 to March 2016. We excluded 10 patients with acute promyelocytic leukemia. This study was approved by the institutional review board of the Ethics Committee and complied with the Declaration of Helsinki. We analyzed the expression of three different LSC markers in gated leukemic blasts was evaluated using 6-color flow cytometry. When over 20% of leukemic blasts were positive for any marker, the sample was defined as positive for that marker. We stratified de novo AML patients into two groups: LSCHigh was defined as positivity for two or three LSC markers, and LSCLow was defined as negativity for all markers or positivity for a single LSC marker. The primary endpoint was overall survival (OS). The secondary endpoint was progression-free survival (PFS). OS and PFS were estimated using the Kaplan-Meier method, and assessed using the log-rank test. Multivariate analysis was performed for OS and PFS.

Results: The median follow-up for patients still alive at the end of the study was 38.9 months (range: 1.5–64.8 months). The median patient age was 60 years (range: 17–78 years). There was no statistical significance between LSCHigh patients (n=30) and LSCLow patients (n=50) in sex, age, laboratory data, NPM1 mutation, or European Leukemia Net karyotype risk group. FLT3 mutation was associated with the LSCHigh group (p=0.003). Three-year OS and PFS were significantly better in the LSCHigh group than in the LSCLow group (Figure 1) (OS: 65.0% vs 18.2%, p <0.001; PFS: 49.3% vs 19.4%, p <0.001). In multivariate analysis controlled for age and karyotype (Table1), being in the LSCHigh group was an independent prognostic factor for OS (hazard ratio: 3.17; 95% CI: 1.64–6.15; p <0.001) and PFS (hazard ratio: 2.25; 95% CI: 1.24–4.08; p=0.007). Being in the LSCHigh group had incremental value for OS compared with the karyotype risk (Harrell's C index: 0.80 vs 0.70; p = 0.028). Moreover, this classification based on LSC marker expression allowed subgroups with unfavorable prognosis to be identified among patients in the intermediate karyotype risk group (3-year OS 54.6% vs 14.5%, p=0.013), as well as those in the favorable karyotype risk group (3-year OS 94.1% vs 50.0%, p=0.021).

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

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MULTIPLE LEUKEMIC STEM CELL MARKER EXPRESSION IS ASSOCIATED WITH POOR PROGNOSIS IN DE NOVO ACUTE MYELOID LEUKEMIA. T. Yabushita1*, Y. Shimomura1, D. Katoh1, Y. Ono1, N. Hiramoto2, S. Yoshioka1, N. Yoneta1, A. Matsuhashi1, H. Hashimoto1, T. Ishikawa1
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Background: Acute myeloid leukemia (AML) is believed to originate from a small population of leukemic stem cells (LSCs). Current chemotherapy regimens target the majority of more mature leukemic blasts, but cannot efficiently eliminate LSCs, resulting in early treatment failure and relapse. Thus, the expression of LSC-specific markers could be used as a predictive factor of clinical outcomes in AML patients. Recently, the clinical impact of individual LSC marker expression could be used as a predictive factor of clinical outcomes in AML patients.

Methods: Ninety consecutive patients diagnosed with de novo AML were included in our study, which was approved by the Ethics Committee and complied with the Declaration of Helsinki. We analyzed the expression of three LSC markers, CD25, CD96, and CD123, in de novo AML patients. The expression of these markers on gated leukemic blasts was evaluated using 6-color flow cytometry. When over 20% of leukemic blasts were positive for any marker, the sample was defined as positive for that marker. We stratified de novo AML patients into two groups: LSCHigh was defined as positivity for two or three LSC markers, and LSCLow was defined as negativity for all markers or positivity for a single LSC marker. The primary endpoint was overall survival (OS). The secondary endpoint was progression-free survival (PFS). OS and PFS were estimated using the Kaplan-Meier method, and assessed using the log-rank test. Multivariate analysis was performed for OS and PFS.

Results: The median follow-up for patients still alive at the end of the study was 38.9 months (range: 1.5–64.8 months). The median patient age was 60 years (range: 17–78 years). There was no statistical significance between LSCHigh patients (n=30) and LSCLow patients (n=50) in sex, age, laboratory data, NPM1 mutation, or European Leukemia Net karyotype risk group. FLT3 mutation was associated with the LSCHigh group (p=0.003). Three-year OS and PFS were significantly better in the LSCHigh group than in the LSCLow group (Figure 1) (OS: 65.0% vs 18.2%, p <0.001; PFS: 49.3% vs 19.4%, p <0.001). In multivariate analysis controlled for age and karyotype (Table1), being in the LSCHigh group was an independent prognostic factor for OS (hazard ratio: 3.17; 95% CI: 1.64–6.15; p <0.001) and PFS (hazard ratio: 2.25; 95% CI: 1.24–4.08; p=0.007). Being in the LSCHigh group had incremental value for OS compared with the karyotype risk (Harrell's C index: 0.80 vs 0.70; p = 0.028). Moreover, this classification based on LSC marker expression allowed subgroups with unfavorable prognosis to be identified among patients in the intermediate karyotype risk group (3-year OS 54.6% vs 14.5%, p=0.013), as well as those in the favorable karyotype risk group (3-year OS 94.1% vs 50.0%, p=0.021).

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

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NEXT GENERATION SEQUENCING TARGETED PANEL FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA. V. Mcclain1*, A.R. Carson1, B.A. Patay1, L. Chamberlain1, C. Chander1, J. Thornes2, T. Stenzel1, J. E. Miller1,2
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Background: Many personalized therapies for acute myeloid leukemia (AML) have been developed targeting specific biomarkers. Unfortunately, the efficacies of these therapies are inconsistent, while the need to determine successful therapies prior to patient relapse is critical. Minimal residual disease (MRD) monitoring can help determine effective treatments and predict potential relapse. While there are now several MRD tests available on the market, most target single or small numbers of biomarkers, which can limit detection of residual AML heterogeneity. Thus, full characterization of a sample may require testing with multiple MRD assays, which can be impractical in a clinical setting. We have developed a target capture-based assay (MyMRD™), which allows characterization of the entire therapeutic AML biomarker repertoire and can inform
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 identify undetected clones.

**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 were also targeted. Enriched 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 (0.01% – 99%). 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%).

**Summary/Conclusions:** The IVS developed MyMRD targeted panel is a sensitive and reliable assay to monitor residual AML driver mutations. The assay is shown to have excellent linearity and a LOD of 0.5% (tenfold lower than the standard CE assay LOD) at >95% of the targeted sites. Additionally, specific mutations of interest, such as those used for residual disease monitoring (e.g. FLT3 ITD), demonstrate LODs as low as 0.1%. The MyMRD assay provides an accurate method for detecting mutations in multiple targets in patients and can be used to effectively stratify patients for therapy and clinical trials.

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IS IT POSSIBLE TO RELIABLY DETECT CLINICALLY-RELEVANT BIALLELIC 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 of the myeloid lineage.

**Aims:** We set ourselves to compare the performance of two different NGS targeted panels on biallelic CEBPA mutations, 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 panel (Illumina) (n=59), and the Ion AmpliSeq AML Community Panel (Theranostics) (n=92) or to NGS (n=81) sequencing, including the TruSight Myeloid panel (Illumina) (n=59), and the Ion AmpliSeq AML Community Panel (Theranostics) (n=92). In 81 additional cases, the gene fusion RUNX1-RUNX1T1 was also targeted. Both panels 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 (0.01% – 99%). 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%).

**Summary/Conclusions:** The IVS developed MyMRD targeted panel is a sensitive and reliable assay to monitor residual AML driver mutations. The assay is shown to have excellent linearity and a LOD of 0.5% (tenfold lower than the standard CE assay LOD) at >95% of the targeted sites. Additionally, specific mutations of interest, such as those used for residual disease monitoring (e.g. FLT3 ITD), demonstrate LODs as low as 0.1%. The MyMRD assay provides an accurate method for detecting mutations in multiple targets in patients and can be used to effectively stratify patients for therapy and clinical trials.

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EXPERIENCE WITH MINIMAL RESIDUAL DISEASE MONITORING IN AML WITH RUNX1-RUNX1T1: A STUDY ON 186 PATIENTS

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**Background:** The cure rate in AML is dependent on patient’s age and performance status, cytogenetics, early blast clearance and sustainable first complete remission. Investigation of minimal residual disease (MRD) is possible by multiparameter-flow cytometry (MFC) or molecular techniques. Recent findings have further depicted a broad spectrum of molecular markers in AML in 99% of pts (TCGA, NEJM, 2013). This broadens the set of targets for MRD monitoring and will hopefully help to better individualize treatment strategies. In this analysis we focused on MRD monitoring in RUNX1-RUNX1T1 positive AML in an unselected cohort.

**Aims:** To understand the clinical use of PCR based MRD monitoring in AML with RUNX1-RUNX1T1 fusion.

**Methods:** Since 2005 und 2017 we investigated a total of 186 intensely treated AML patients with RUNX1-RUNX1T1 fusion, 130 of them diagnosed at our laboratory and 56 with follow up samples available. 1448 individual samples were analyzed during the course of disease. We applied quantitative real-time PCR to detect RUNX1-RUNX1T1/ABL ratios. Complete molecular remission (CMR) was defined as one valid qPCR ratio of 0, while low MRD was assigned to patients with a >0 but <0.01 ratio and high MRD was assigned to all patients with a ratio above 0.01. As a comparator log fold change to baseline was independently assessed. Median age was 51 years.
(18-83 years). All patients were treated with standard induction and consolida-
tion protocols.

Results: Median time between two investigations was 2.8 months (range for all 0.1-115 months). A complete molecular remission was reached in 90/130 pts (69%) after a median of 5 months. 19/130 (14.6%) pts reached low level MRD and 20/130 (15.4%) high level MRD. Median event free survival (EFS) of patients with CMR was not reached (EFS at 2 years 82%). 16 (18%) of those patients relapsed in the course of follow up with a median time to relapse of 12.7 months (range 4.1 to 38.3 months). Median EFS for MRD low and MRD high patients was 18.4 months and 10.8 months respectively (all 3 groups, p<0.0001). For patients with CMR, rising MRD levels accurately predicted relapse with a median latency of 5.5 months from loss of CMR to relapse. We next used the widely accepted log fold change from baseline to define high and low risk patients in our cohort. 123/130 (95%) patients reached a >3 log fold reduction in RUNX1-RUNX1T1/ABL ratio within the first 200 days following first diagnosis. Median EFS for those patients was not reached (EFS at 2 years 66%). The 7/130 (5%) patients with a <3 log fold reduction had a median EFS of 14.7 months (2 groups, p=0.017). A total of 59/185 patients received allo-
geneic 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 per-
formed in RUNX1-RUNX1T1 AML outside of clinical studies. Defining MRD
levels by RUNX1-RUNX1T1/ABL ratios resulted in a better classifier for high
and low risk patients than log fold change. However, despite CMR 16/90 (18%)
patients relapsed with a maximum time from first achievement of CMR of 38.3
months. We conclude that 1) MRD monitoring could serve to guide BMT deci-
sions in RUNX1-RUNX1T1 positive AML, 2) allogeneic BMT can rescue the
majority of relapsed patients and 3) molecular monitoring can reliably identify
patients with high risk for relapse.
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NUMBER OF TP53 ABNORMALITIES AND THEIR CLINICAL RELEVANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELOPROLIFERATIVE SYNDROMES

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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 884 (70%) patients, data on therapy with sufficient follow up and response evaluation was available, with 494 (35%) patients receiving therapy with hypomethylating agents (HMAs) and 373 (27%) with chemotherapy regimens. A total of 384 mutations in TP53, involving 208 unique mutations, were detected among 300 (21%) patients with R273H, R248W, Y220C and R175H being the most prevalent. Overall frequency of TP53 mutations was higher among patients with MDS (25%, n=146) compared to AML (19%, n=154) (p=0.012) with 251 patients had 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 abnormality, 169 (12%) patients having 1 TP53 abnormality, 169 (12%) patients having 1 TP53 abnormality. Patients with multiple detectable TP53 mutations were less likely to have co-occurring chr17 abnormalities (79% vs 22%, OR 0.28, CI 0.15-0.50, p=0.03). Median follow up was 8.6 months (range 0-167 months). Presence of a TP53 mutation adversely impacted OS (MDS: 12 vs 111.7 months, HR=5.98, CI 14.28-8.35, p<0.001; AML: 5.3 vs 16.9 months, HR=2.81, CI 2.26-3.50, p<0.001). Increasing number of TP53 abnormalities negatively impacted OS of patients with AML (Figure 1A) but not that of patients with MDS (Figure 1B). No difference in survival was observed between patients with two TP53 mutations and those with TP53 mutation+deletion (p=0.730). Presence and number of TP53 mutations did not predict for response (OR: 60 vs 63% p=0.498; CR: 34 vs 36%, p=0.695) to HMAs, but was associated with significantly lower likelihood of response to intensive chemotherapy (OR: 41 vs 86%, p<0.001; CR: 33 vs 75%, p<0.001).

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

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VADASTUXINAB TALIRINE PLUS HYPOMETHYLATING AGENTS: A WELL-TOLERATED REGIMEN WITH HIGH REMISSION RATE IN FRONTLINE OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA: RESULTS FROM A phase 1/2 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. Deep remissions are 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). Vadaastuxinab talirine (2CGN-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 phase 1/2 study (NCT01903239) was designed to evaluate the safety, tolerability, PK, and antileukemic activity of 33A in combination with an HMA.

Methods: Eligible patients (ECOG status 0-1) had previously untreated CD33-positive AML. One dose of 33A (10 mcg/kg) was administered outpatient IV every 4 weeks on the last day of HMA (azacitidine or decitabine [5-day regimen], standard dosing). CRi required either platelet count of ≥100,000/µL or minimal residual disease (MRD) negativity (F Ravandi, MD, unpublished data, Jan 2017). Vadaastuxinab talirine (2CGN-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.

Figure 1.
ACUTE MYELOID LEUKEMIA WITH INTERMEDIATE-RISK CYTOGENETICS AND A FAVORABLE GENOTYPE: PROGNOSTIC FACTORS AND RESULTS IN PATIENTS TREATED ACCORDING THE SPANISH CETLAM PROTOCOLS

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

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

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

Results: Two hundred and twenty-one patients were analyzed. Median age of the series was 54 years (range 18 to 72). 152 patients had an age up to 60 years and 69 (31%) were older. Median WBC count was 19x10^9/l (range 0.55-282). One hundred eighty-two patients had a normal karyotype and it was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and was abnormal in 34 (5 patients no metaphases).

No significant differences in the main clinical or biological parameters in these two groups. The CR rate in the overall group was very high (92%) without significant differences between the two molecular groups. Chemo-resistance was observed in only 2 patients of the NPM1+/FLT3-ITD- group (1%). 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 allogeic transplant. One patient died in CR in the HDAC group, another in the autologous transplant group and 7 in the allo-HCT group (p<0.0001). 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).

Figure 1.

Summary/Conclusions: Patients with primary AML, IR cytogenetics and FLT3 have a good outcome. Best results are achieved in patients with CEBPα+/FLT3-ITD-, particularly if age is up to 60 years. In this subset, OS at 8 years is 96±7%, comparable to current results achieved in acute promye-
locytic leukemia. Patients above 60 years treated intensively may achieve a long term survival of more than 50%. Chemotherapy without subsequent transplantation is a valid option. MRD monitoring after treatment has to be taken into account since in the subset of patients analyzed this was an independent prognostic factor for EFS.
Results: 24 pts have been enrolled to date and 17 are evaluable for response. The median age was 66 years (range, 46–79) with 58% male pts and 25% with high-risk cytogenetics (by SWOG). 50% (12/24) were pts with secondary AML (sAML), half of whom had prior hypomethylating chemotherapy (50%; 6/12). This study had a rolling safety run-in and the first 3 pts had no DLT, allowing enrollment to proceed. Common Gr 3/4 AEs included febrile neutropenia (47%), pneumonia (20%), cancer (13%) and non-fatal respiratory failure (13%), 2 pts died of sepsis within 60 days. The remission rate (CR/CRi) was 12/17 (71%); CRi/CR 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 secondary AML in Phase 1 (DeAngelo, EHA 2016); no accumulation or evidence of drug-drug interactions were apparent. The median E-sig elimination rate at baseline was 29% (range, 2-67%) of blasts in the bone marrow.

Summary/Conclusions: The addition of a novel E-selectin antagonist, GM-1204 to anthracycline- and daunorubicin 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.

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A PHASE 2 STUDY OF GLASDEGIB (PF-04449913) IN COMBINATION WITH CYTARABINE AND DAUNORUBICIN IN UNTREATED PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA OR HIGH-RIK MYELOGENOUS SYNDROME


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Background: Glasdegib, a selective, once-daily (QD), oral Smoothened (SMO) inhibitor, demonstrated significant improvement in overall survival (OS) when used in combination with low-dose cytarabine (LDAC) vs LDAC alone in a randomized (2:1) open-label trial in 132 patients (pts) not suitable for induction chemotherapy (ICT). 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. This was the key safety and endpoint.

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

Results: All Pts: As of 1 Dec 2016, 71 pts (66 AML, 5 MDS) were enrolled and 69 pts received glasdegib and ICT (2 pts not treated due to ineligibility). Among MDS pts (47 de-novo vs secondary), 20% had favorable, 32% intermediate (int)-II, 21% int-II and 26% adverse cytogenetic abnormalities (1 pt not eval).

| Summary/Conclusions: Although the CR rates do not appear to be higher than those reported historically for AML pts receiving ICT, the mOS for AML pts >60 yrs stratified by subgroup compares favorably by adding glasdegib. Although the CR rates do not appear to be higher than those reported historically for AML pts receiving ICT, the mOS for AML pts >60 yrs stratified by subgroup compares favorably by adding glasdegib. The effect of glasdegib on the leukemia stem cells. The combination of glasdegib with ICT was well tolerated, with a safety profile consistent with that in AML pts receiving standard ICT. Further studies are warranted.

CM942 IS A NEW SMALL MOLECULE THAT TARGETS SET-PP2A INTERACTIONS AND INHIBITS GROWTH OF ACUTE MYELOGENOUS 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 (PAD), depends on its interaction with SET. FT720 is a relatively nontoxic 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 (10). By 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 PP2Ac, and that treatment with CM942 effectively disrupted this association. Furthermore, CM942 had a caspase-dependent pro-apoptotic effect, and decreased phosphorylation 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 FTY720 was somewhat more active than CM942 in primary samples of AML, fewer cytotoxic effects were observed after CM942 treatment in peripheral blood from healthy donors. Further experiments would be necessary to confirm the in vivo anti-tumor activity of CM942 in AML models. New compounds have been developed for the treatment of AML, although few have been translated into clinical practice; nevertheless, it is unlikely that any of these compounds, when used alone, will cure the disease, for which the most effective combination therapy. 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 clone of AML. Assuming that most of the mutations are heterozygous and considering the karyo-type at diagnosis, mutations of the biggest clusters were present in every cancer cell and the mutations of the smaller clusters in proportionately smaller fractions. Based on the clustering information we were able to model the potential clonal hierarchies. Using a combinatorial approach, clonal models of the clonal architecture (2n-1; n= number of clusters) were present at the time of diagnosis and in which order they evolved (Figure 1). Through comparison of clusters from diagnosis and relapse clonal selection can also be detected and via modelling the most likely clonal architectures can be identified. By assigning our 64 identified leukemia-specific mutations to the defined clusters we can now track the different clusters/clones in phenotypically distinct subpopulations and during xenotransplantations by targeted sequencing. 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.

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.

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

Summary/Conclusions: Maintaining SD during AZA or CCR Tx was associated with significantly lower OS and EFS. The median OS were 18 months and 12 months, respectively; the 2-year EFS rate was 44% in both arms (P=0.91). 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 groups were similar with respect to pretreatment characteristics analyzed, including age, cytogenetics, and ENS risk. No differences were observed in CR/CRp rates, EFS or OS between the two groups. However, among pts <50 years of age, the median EFS for pts who received FIA (n=36), CIA (n=28) and IA (n=34) was not reached. 10-months and 2-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 have similar efficacy in younger pts with newly diagnosed AML, although FIA is associated with a better toxicity profile. FIA may improve outcomes compared to IA in pts <50 years of age.

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/CRp rate was similar between the two arms (80% for CIA vs 82% for FIA; P=0.84). CR was achieved in 72% and 74% in the CIA and FIA arms, respectively. MRD negativity rates at remission by 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 10 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 cytogenetic mutations, or ENS risk group. CIA was generally associated with lower 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 were excluded from this analysis. The two groups were similar with respect to pretreatment characteristics analyzed, including age, cytogenetics, and ENS risk. No differences were observed in CR/CRp rates, EFS or OS between the two groups. However, among pts <50 years of age, the median EFS for pts who received FIA (n=36), CIA (n=28) and IA (n=34) was not reached. 10-months and 2-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).

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Table 1.
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.

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P211
NIVOLUMAB MAINTENANCE THERAPY FOR PATIENTS WITH HIGH-RISK ACUTE MYELOID LEUKEMIA IN REMISSION
T. Kadia1,*, H. Kantarjian1, E. Jabbour2, F. Ravandi1, N. Daver1, P. Cardenas1, M. Brandt1, M. Konopleva1, J. Cortes1
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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 maintaining CR4 with conventional cytotoxic 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 relapse.

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

Methods: AML pts ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Pts should be within 12 months of achieving CR, have PS ≤2, and adequate organ function. Pts were treated with nivo 3mg/kg IV every 2 weeks for 6 months. 1 cycle was 4 weeks. After 6 months, nivo could be given every 4 weeks until 12 months on study, and then every 3 months until relapse. All pts had baseline cytogentic 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 mutational 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 patient had grade 3 pneumonitis leading to hypothyroidism, treated successfully with steroids and thyroid hormone supplementation, who continues on treatment. 1 patient 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 for 1 pt. Another pt continues on the regimen. 2 pts had grade 3 possible pneumonitis treated successfully with steroids

Figure 1.

P212
HIGHER EXPRESSION OF LONG NON-CODING RNA KIAA0125 IS ASSOCIATED WITH CHARACTERISTIC CLINICAL AND BIOLOGICAL FEATURES AND IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA
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Background: Long non-coding RNAs (IncRNAs) are non-protein coding RNAs longer than 200 nucleotides. Recently, a number of IncRNAs have been shown to play important roles in cancer biology. IncRNA KIAA0125 is one of the 11 genes in an expression signature significantly associated with prognosis in cytogenetically normal acute myeloid leukemia (AML) patients as shown in our previous report. It is also among another set of 17 leukemia stem cell (LSC) genes, identified through xenotransplantation model in NSG mice, which predict inferior treatment response in AML.

Aims: KIAA0125 gene is localized on chromosome 14q32.33; its functions remain unexplored. One study reported that it might be involved in neurogenesis including induction of astrocitosis, 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 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,

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RUNX1 mutation, MLL-PTD, WT1 mutation, and TP53 mutation (p<0.011).

Summary/Conclusions: Higher expression of KIAA0125 in AML patients was correlated with mutations of RUNX1, DMNT3A, 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
O. Pérez-López1, T. Caballero-Velázquez1, I. Álvarez-Laderas1, P. Hernández-Diaz1, A. M. Márquez-Mattito1, J. González-Campos1, R. Morales-Camacho1, C. Prats-Martín1, M.T. Vargas-de los Monteros1, R. Bernai-Ruiz1, J.A. Pérez-Simón1
Virgen del Rocío University Hospital, Sevilla, Spain

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

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

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

Results: Out of the 67 patients [34 men/33 women; median age 54 (0-78)], 58 (88.5%) have SC at diagnosis, 37.9% of them (n=22) achieved complete remission (CR) with a negative minimal residual disease (MRD) and 77.8% (7/9) among patients with SC (p=0.03). Among patients who obtained CR with a negative MRD (n=29), no one suffered 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 subgroup of patients with different prognostic among those in the intermediate risk group by genetics/molecular assays.

P214
POST-REMISSIONAL AND PRE-TRANSPLANT ROLE OF MINIMAL RESIDUAL DISEASE DETECTED BY WT1 IN ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE COHORT STUDY
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1Department of Hematology, 2Department of Pathology, 3Bone Marrow Transplant Center, University-Hospital Città della Salute e della Scienza, Torino, Italy, 4Unit of Clinical Epidemiology, University-Hospital Città della Salute e della Scienza and CPO Piemonte, Torino, Italy

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

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

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

Methods: The present retrospective cohort study included adult patients with untreated AML consecutively diagnosed between 2004 and 2014 in the Hematology Unit of the University-Hospital Città della Salute e della Scienza of Torino, Italy. The study was approved by the Ethical Committee and was registered at www.clinicaltrials.gov as NCT02714790. Among 255 enrolled patients, MRD was investigated in those in first complete remission (CR) with an available at diagnosis and at two further time-points: after induction (n=117) and prior allo-HCT (n=65). Patients with baseline WT1 <250 copies were excluded. AML 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 (CRi) and survival 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 (CR 1.16, 95% CI 0.90-1.50, p=0.244). OS and DFS were significantly shorter in patients in first CR with >350 WT1 copies after induction compared to those with ≤350 (OS 17 vs 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 (NR=0.4037) for DFS. Before allo-HCT, patients with WT1 >150 copies (n=18) had a significantly higher CIR compared to those with WT1 ≤150 (n=47), HR 4.61; 95% CI 1.72-12.31, p=0.002.

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

Figure 1.
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 mAML treated with enasidenib, and is likely due to its suggested mechanism of action, myeloblast differentiation.

Aggressive Non-Hodgkin lymphoma - 1st line

P216
Abstract withdrawn.

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


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

Aims: Our work aims to describe clinical presentation and outcome of IVLBCL patients treated in French LYSA centers between 2000 and 2016.

Methods: All LYSA centers were asked to report and update clinical data about IVLBCL patients treated. No central pathology review was performed for the present study, but all cases were classified by LYSA pathologists. Local investigators reported disease characteristics and updated patients’ outcome (clinical exam, CT scan at baseline, CT response evaluation and outcome).

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

Pathological features (including cell of origin characterization, C-MYC expression, adhesion protein expression level) investigation is ongoing and will be presented at the time of the meeting.
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.

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

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

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

Results: Median age was 81 years (80-88), 51.8% of men. Proportion of pts ≥85 was 14.3%, with PS ≥2 (ECOG) 34.0%, with higher LDH 64.3%, with high β2microglobulin (HR 2.2, p 0.05), low albumin (HR 1.9, p 0.05) and PS ≥2. There were observed 11 treatment related deaths (6 cardiac toxicity related, resp. There were 21 pts with CR or PR have significantly better OS median (as resp. 6 and more cycles were administered in 71.4%, 63.1% and 58.5% resp. Following proportion of pts. received >80% (>50%) of original CHOP dose. for A it was 57.1% (76.1%), >80% (>50%) of original CHOP dose. for Cyclophosphamide it was 66.7% (>50%) of original CHOP dose. for Adria mycin – A - 50 mg/m2) or R-MiniCHOP (minICHOP) (CF 400 mg/m2, A 25 mg/m2) or any other dose between CHOP and miniCHOP). There were 21 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 (18-60, 61-70 and >70 years), as compared with 2.7, 18.7 and 19.5 in the last period. The application of CT+RT increased exclusively among pts age 18-60. More specifically, the proportions for the three age groups were 26, 18 and 4% in the first period, as compared with 60, 10 and 4% in the last period. The use of 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 therapy and RT alone in the last period, respectively. Five-year RS only improved for pts age 18-70 (Figure 1). Five-year RS (95% confidence intervals) was 22% (16%>30%), 13% (7%>22%), and 3% (1%>10%) in the first period for the three age groups, as compared with 56% (47%-64%), 35% (28%-43%) and 6% (2%-13%) in the last period. A multivariable survival model confirmed the adverse effect of older age on excess mortality and an improvement of survival over time. However, when information on treatment was added to that model, the effect of period lost statistical significance. This suggest that treatment contributed to the improved survival over time. Older age remained a predictor of poor prognosis.

Figure 1.

Summary/Conclusions: The incidence of PCNSL steadily increases among
pts >60 years, which might in part be related to improved diagnostic practices among the elderly. Over time, RS increased over the past decades for pts >70 or below. This is largely explained by the increased use of intensive therapy over time. Although the use of CT alone gradually increased among pts >70 years, their survival is still poor. Therefore, there is an urgent need to design specific trials for elderly PCNSL pts to improve their survival.

P220

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

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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 multistage etiopathogenesis DLBCL.

Aims: The purpose of this study was evaluation of clinical features and results of treatment of diffuse krepnokletochnoy lymphoma associated with hepatitis C in comparison with a control group of patients with diffuse large lymphoma without viral hepatitis markers.

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

Results: Patient’s age ranged from 21 to 76 years (median was 47 years) in DLBCL+C; ranged from 23 to 81 years (median 61) in DLBCL-C (p=0.02). The male: female ratio was 1:1.3 in patients with DLBCL+C; 1:1.7 in the group DLBCL-C. Stage I and II were in 11% patients with DLBCL+C, and 48% patients with DLBCL-C; ranged from 23 to 81 years (median was 61) in DLBCL-C (p=0.02). Extranodal localization of extranodal 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 CARDIOTOXICITY IN MALIGNANT LYMPHOMA

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: Doxorubicin is a cornerstone of curative therapy and 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 cardio toxicity 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. The study was stopped on May 30, 2016. 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 42 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 7.4 mL, in mean stroke volume (SV) (p=0.02). However, from the acute MR to follow-up MR we found a significant decline in SV (p=0.02). There was no difference in SV from baseline to follow-up (p=0.7). The acute changes in LVEDV did not predict LVEF declines from baseline to follow-up (Figure 1).

Figure 1.

Summary/Conclusions: Our preliminary show that cardiac MR can be used for detection of declining LV function 1 year after 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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1Department of Internal Medicine, Korea University School of Medicine, 22nd Congress of the European Hematology Association

Background: Doxorubicin is a cornerstone of curative therapy and 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 cardio toxicity 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. The study was stopped on May 30, 2016. 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 42 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 7.4 mL, in mean stroke volume (SV) (p=0.02). However, from the acute MR to follow-up MR we found a significant decline in SV (p=0.02). There was no difference in SV from baseline to follow-up (p=0.7). The acute changes in LVEDV did not predict LVEF declines from baseline to follow-up (Figure 1).

Figure 1.

Summary/Conclusions: Our preliminary show that cardiac MR can be used for detection of declining LV function 1 year after 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.
2Department of Internal Medicine, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic Of

Background: 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 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 was detected in the other 85 (56%). Detection of asymptomatic relapse was identified in 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 CT was not improve post-relapse survival in the relapsed aNHL patients. In addition, the interval of surveillance CT (3 or 6 months) did not affect survival. No subgroups were identified that favored the use of surveillance CT to detect relapse. Additionally, we analyzed the impact of surveillance CT in patients with refractory or relapsed aNHL who achieved CR after salvage chemotherapy (CR2). Of 315 aNHL patients relapsed/refractory to frontline chemotherapy (144 refractory and 171 relapsed patients), 99 patients achieved CR after salvage chemotherapy (18 patients relapsed/refractory to frontline chemotherapy with or without surveillance CT).

Results: 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 CT was not improve post-relapse survival in the relapsed aNHL patients. In addition, the interval of surveillance CT (3 or 6 months) did not affect survival. No subgroups were identified that favored the use of surveillance CT to detect relapse. Additionally, we analyzed the impact of surveillance CT in patients with refractory or relapsed aNHL who achieved CR after salvage chemotherapy (CR2). Of 315 aNHL patients relapsed/refractory to frontline chemotherapy (144 refractory and 171 relapsed patients), 99 patients achieved CR after salvage chemotherapy (18 patients relapsed/refractory to frontline chemotherapy with or without surveillance CT).

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

Table 1

<table>
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<th>Characteristic of relapse according to method of detection</th>
<th>CR achieved</th>
<th>CR not achieved</th>
<th>p-value</th>
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<tr>
<td>Time to relapse, months</td>
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<tr>
<td>3 months</td>
<td>45%</td>
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<td>9 months</td>
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Bone marrow failure syndromes incl. PNH - Biology

P226
IDENTIFICATION OF A NOVEL GERMINE MECOM / EVI1 VARIANT THAT RUNS IN A PEDIGREE WITH RADIOUNAR SYNOTOSIS AND AMEGAKARYOCYTIC THROMBOCYTOPENIA AND PREDICISES TO ADULT ONSET MYELOID MALIGANNAY

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Background: Radioulnar synotosis and amegakaryocytic thrombocypenia (RUSAT, one of the rare bone marrow failure syndromes, is caused by a point mutation in HOXA11. In three simplex patients, de novo missense variants in MECOM have recently been reported as an alternative cause in individuals with RUSAT. MECOM, identified as a common ecotropic viral integration site 1 (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 radioulnar synotosis, incompletely penetrant congenital thrombocypenia, hearing impairment due to dysplastic middle ear bones, patellar hypoplasia, and hand and foot dysmorphism.

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 pathogenic and predicted to be deleterious, using a combination of MetaLR, (ii) reported 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 highly conserved cysteine residue in C2H2 zinc finger motif 9 in the C-terminal zinc finger domain of MECOM. This residue is crucial for the tetrahedral coordination of a zinc ion stabilizing the zinc finger conformation and thus, is essential for DNA binding of the C-terminal zinc finger domain.

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

[BZ and DS contributed equally to this work].

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LOSS OF THE HOMOLOGOUS RECOMBINATION GENE RAD51 LEADS TO FANCONI ANEMIA-LIKE SYMPTOMS IN ZEBRAFISH

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1Department of Haematology, University of Cambridge, 2Wellcome Trust Sanger Institute, 3Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, 4NHS Blood and Transplant, Cambridge, United Kingdom

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 indispensable homologous recombination protein, necessary for strand invasion and crossing over. It has been extensively studied in prokaroytes and lower eukaryotes. 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. Additional lines we aim to utilize contain useful genetics and translucency of zebrafish to dissect the role of rad51 in hematopoesis and to explore the molecular basis of Fanconi anemia pathogenesis.

Methods: Zebrafish carrying homozygous loss of function mutations in rad51
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. Co-mutation of p53 was able to rescue the embryonic and adult hematopoietic defects seen in the single mutants, but led to early tumor development in the adult double mutants. We further establish that prolonged inflammatory stress can exacerbate the hematological impairment, leading to an additional decrease in kidney marrow cell numbers linked to excess p53 expression (Figure 1).

Figure 1. Example image of a p53, rad51 double mutant fish with a tumor behind the eye (A). Histological analysis showed the tumour to be a malignant peripheral nerve sheath tumor (B). The scale bar is 500 and 10µm respectively.

**Summary/Conclusions:** We demonstrate that zebrfish lacking functional rad51 is viable and develop symptoms resembling FA. These findings strengthen the assignment of RAD51 as a Fanconie 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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1Department of Haematology, University Hospitals Bristol NHS Trust, 2School of cellular and molecular medicine, University of Bristol, Bristol, 3BRC Blood Theme and BRC/NIHS Translational Molecular Diagnostics Centre, John Radcliffe Hospital, 4Molecular Haemato-Medical Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom

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

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

**Methods:** Genomic DNA samples were analysed for sequence variants using the Oxford Red Cell Panel, a panel of 33 genes previously associated with human red cell disorders. Sanger sequencing was used to confirm the new TERC variant. Telomere lengths were performed at the Laboratory for Molecular and Cellular Medicine, University of Bristol. Genomic DNA samples were analysed for sequence variants using a newly identified TERC variant, short telomere length and a relatively mild haematological phenotype that is largely restricted to red cells. This emphasises the phenotypic heterogeneity associated with TERC variants, justifies the rationale of screening multiple genes simultaneously and suggests that TERC variant could potentially underlie a broader range of unexplained heritable blood cell abnormalities.

**Summary/Conclusions:** This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild hematological phenotype that is largely restricted to red cells. This emphasises the phenotypic heterogeneity associated with TERC variants, justifies the rationale of screening multiple genes simultaneously and suggests that TERC variant could potentially underlie a broader range of unexplained heritable blood cell abnormalities.

P229

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

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**Background:** X-linked Dyskeratosis congenita (X-DC) is an inherited syndrome caused by mutations in the DKC1 gene that encodes for the dyskerin nuclear protein. These mutations reduce the telomerase activity leading to premature telomere length attrition. Several organs can be affected in these patients, although the bone marrow failure (BMF) is the main cause of death in X-DC patients (more than 70% of cases). So far, the only curative treatment for BMF in DC patients is hematopoietic stem cell (HSC) transplantation. However, risks derived from conditioning regimes and the difficulties to find a compatible donor suggest that gene therapy may constitute a promising alternative in treating DC patients.

**Summary/Conclusions:** This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild hematological phenotype that is largely restricted to red cells. This emphasises the phenotypic heterogeneity associated with TERC variants, justifies the rationale of screening multiple genes simultaneously and suggests that TERC variant could potentially underlie a broader range of unexplained heritable blood cell abnormalities.
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 somatic cell reprogramming using different anti-DKC1 shRNA and tissue factor (TF), which explains their involvement in thrombosis in the context of PNH patients under eculizumab. 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. We compared the results of the procoagulant activity, in order to check, if the antithrombotic activity of the eculizumab could be in part explained by its interaction with the EVs.

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 principles of Helsinki and approved by the local Ethics Committee. Informed consent was obtained for each patient. The aim was to measure, by flow cytometry, the production of EVs in patient's platelet-free plasma (PFP) before the start of eculizumab, 4 weeks and after 11 weeks of treatment. We also assessed the procoagulant activity in PFP by STA®-Procoag-PPL assay and by thrombin generation assay (TGA). A more sensitive version of TGA was also performed to study the procoagulant profile induced by the EVs (use of EVs pelleted from PFP). We used mixed-effects linear regression (R 3.1.2 with nlme package) with logarithmic transformation for flow cytometry results. We compared the results after 4 weeks or 11 weeks of treatment against the inclusion value. Results: We observed a decrease in platelet EVs on the eculizumab treatment (p<0.05). STA®-Procoag-PPL assay showed a decrease of the procoagulant profile induced by procoagulant phospholipids (PL) with the treatment. These results were not confirmed by TGA on PFP, due to a lack of sensitivity. By this way, we performed a more sensitive version of TGA that allows to observe variation in the procoagulant profile induced by the EV with the eculizumab (p<0.05).

Summary/Conclusions: Eculizumab has an impact on the amount and the procoagulant profile induced by the procoagulant PL and the EVs. The anti-thrombogenic performance of the eculizumab can be in part explained by its action on EVs.

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STUDY OF EXTRACELLULAR VESICLES ROLES IN THE PATHOPHYSIOLOGY OF THROMBOSIS IN PAROXYSMAL NOCTURNAL HEMOGLOBINOPATIA PATIENTS DURING ECULIZUMAB TUMORUBAM: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY
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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 principles of Helsinki and approved by the local Ethics Committee. Informed consent was obtained for each patient. The aim was to measure, by flow cytometry, the production of EVs in patient's platelet-free plasma (PFP) before the start of eculizumab, 4 weeks and after 11 weeks of treatment. We also assessed the procoagulant activity in PFP by STA®-Procoag-PPL assay and by thrombin generation assay (TGA). A more sensitive version of TGA was also performed to study the procoagulant profile induced by the EVs (use of EVs pelleted from PFP). We used mixed-effects linear regression (R 3.1.2 with nlme package) with logarithmic transformation for flow cytometry results. We compared the results after 4 weeks or 11 weeks of treatment against the inclusion value. Results: We observed a decrease in platelet EVs on the eculizumab treatment (p<0.05). STA®-Procoag-PPL assay showed a decrease of the procoagulant profile induced by procoagulant phospholipids (PL) with the treatment. These results were not confirmed by TGA on PFP, due to a lack of sensitivity. By this way, we performed a more sensitive version of TGA that allows to observe variation in the procoagulant profile induced by the EV with the eculizumab (p<0.05).

Summary/Conclusions: Eculizumab has an impact on the amount and the procoagulant profile induced by the procoagulant PL and the EVs. The anti-thrombogenic performance of the eculizumab can be in part explained by its action on EVs.
Background: Mortality following HSCT in SAA pts over the age of 40 is reported to be in the order of 50%, without taking into account long term sequelae such as chronic GvHD, known to be more frequent in older patients. This has prompted international guidelines to recommend first line immunosuppressive therapy above 40 years of age. The question is whether this is still true in 2017.

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

Methods: We used the WPSSA-EBMT registry, and identified 748 pts aged 40 years or more, with acquired SAA, grafted between 2001 and 2015. We divided pts in 2 transplant eras:2001-2009 (n=327) and 2010-2015 (n=407). In the more recent period (2010-2015) pts were older (53 vs 49 year, p<0.01), were more often grafted from 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 in the conditioning , did significantly better than pts not receiving Campath (65% vs 54% p<0.01); similarly survival of patients with ATG was superior 59% vs 41% compared to patients not receiving ATG (p=0.01). When pts receiving either Campath or ATG (n=564) were compared to patients not receiving either (n=161), the difference in survival was 61% vs 41% (p<0.0001), and this was significant also in multivariate analysis. Combined primary and secondary graft failure was reduced from 16% to 12% in the two time periods (p=0.02), acute GvHD grade II-IV was reduced from 15% to 11% (p=0.05) and chronic GvHD was also reduced from 32% to 26% (p=0.03). Infections remain the leading cause of death in both transplant eras (18% and 22% respectively), followed by GvHD (5% and 4%) and graft failure (5% and 2%), whereas PTLD have been reduced from 3% to 0.5% (Figure 1).

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

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

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 multilineage (63) MF. 48 (64%) were classified as having an acquired MF; 27 (30%) were diagnosed with a congenital MF (FA 11, Dissekratosis Congenita 5, Severe Congenital Neutropenia 6, Blackfan-Diamond Anemia 3, Congenital Amegakaryocytic Thrombocytopenia 2), and the remaining 13 patients (14%) were found to have 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 P13KCD, 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.

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

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 significant adverse events and the drug has been well-tolerated. A few mild injection site reactions have been recorded but these appear to diminish with time. There has been no evidence of the formation of neutralising antibodies.

Summary/Conclusions: It currently appears that treatment with Coversin is safe and effective in controlling hemolysis in PNH and that patients are capable of self-administering the drug. Coversin may be an effective alternative for patients with PNH who prefer the independence of self-administration. The relatively short dose interval may also help to reduce breakthrough events due to trough levels of drugs administered at two weekly intervals or longer.
Chronic lymphocytic leukemia and related disorders - Biology 1

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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 19% of the inherited component of CLL.

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

Methods: We investigated 516 CLL patients of European descent who were compared to 8,920 ethnically matched, non-cancer population controls. CLL cohorts included 235 CLL patients from DFCI (128 previously reported, 107 unpublished exomes), and 281 CLL patients enrolled on the CLL8 trial of the German CLL Study Group (WES data reported previously). An additional 130 CLL patients in an extension cohort included 24 from our published whole-genome sequencing study and 106 from an early publication of the ICGB. 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 controls available for the association analysis. We further controlled for residual 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 1.2-6.1, p=5.8x10⁻⁷ and OR 1.6, CI 1.3-2.0, p=1.4x10⁻⁴, respectively). CDK1 variants were observed in 8 of 516 CLls and of 78,920 controls (1.6% vs 0.3%, OR=5.8, 95% CI 1.2-6.1). One recurrent missense variant, CDKT 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 124 rare variants in 1296 controls carrying 292 rare variants (21.7% vs 6.9%, OR=21.7, 95% CI 14.5-30.3, p=5.8x10⁻⁷). The majority of recurrent rare variants in ATM were non-synonymous missense variants, with L2307F one of 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 2.0% (3 out of 149) of the L2307F 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 the presence of TREs from raw PRO-seq data, allowing for identification of functional elements that influence nearby target genes.

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

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DIFFERENTIAL ENHANCER TRANSCRIPTION ASSOCIATED WITH RISK ALLELE GENOTYPE IN CLL

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

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

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

Results: Our gene expression analysis demonstrated 6 significant SNP-gene associations: rs674313 (6p21.3) with HLA-DOA1 (p <0.0001), rs8720701 (6p25.3) with HLA-DOA1 (p=0.01), rs4777184 (15q23; proxy for rs7176508) with TLE3 (p<0.0001), rs783540 (15q25.2) with CEBP1 (p=0.01), rs305088 (16q24.1; proxy for rs305061) with CD14NB/EMC3 (p=0.03) and rs498232 (19q13.32; proxy for rs11083846) with FKR P (p<0.0001). Two associations were successfully validated in a completely independent gene expression replication analysis (n=54): rs674313 with HLA-DOA1 (p<0.0001) and rs4777184 with TLE3 (p=0.0116). To annotate candidate regulatory elements, we evaluated transcription level at or near all six significant functional variants in a direct functional analysis in a cohort of 12 CLL samples. Transcription level at or near 3 SNPs (rs674313, rs4777184, rs305088) correlated with genotype in a dose dependent manner. When we expanded the analysis to the entire region of LD around each SNP, we were able to demonstrate a dose-dependent effect in all SNPs in E-2071.

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

Results: Out of the cohort positive for TP53mut, 72/200 patients (36%) harbored single dominant TP53mut without del(17p). We selected 43 of these cases with variant allele frequency (VAF) ≤10% for CytoScan analysis to explore the potential presence of 17p cn-LOH. In 42% (18/43) of the cases, cn-LOH in 17p locus was detected in a proportion of CLL clone correspondingly to the TP53 VAF (median TP53 VAF 59.4%, range 12.9–99.9%). In 3/43 cases, heterozygous deletion previously undetected by FISH was newly revealed. Thus, the truly monoallelic mutations with concomitant 17p deletion were assessed by FISH and Sanger sequencing. To evaluate whether both alleles of the TP53 gene would have escaped detection by Sanger sequencing. Therefore ten of the 26 patients were identified in whom the TP53 mutation status (exons 2-11) sequence analysis was performed by next generation sequencing (e.g. Array (Affymetrix) platform which allows the detection of copy number alterations (CNAs), down to 100 kb in size, and the EU Horizon2020 project No. 692298, and MEYS CEITEC2020 LQ1601.

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

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

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Background: 5-cell chronic lymphocytic leukemia (CLL) exhibits a highly heterogeneous clinical course, with overall survival rates varying from several months to decades. Mutation status of the IGHV genes and specific genomic abnormalities, such as deletion of 11q22 or loss of the 13q14 region provide prognostic information. However, more importantly deletion of 17p and/or the presence of a TP53 mutation, which are both associated with a poor prognosis identified in CLL patients with the highest risk category. Recently clinical trials with tyrosine kinase inhibitors such as ibritinib andidelisib have demonstrated good responses in CLL patients with 17p deletion and/or TP53 mutations. In many studies interphase fluorescence in situ hybridization (FISH) for the detection of 17p deletions and Sanger sequencing of exons 4-10 of the TP53 gene are used as standard classification tools in patients belonging to the highest risk group, due to absence of information regarding the heterozygosity status and low sensitivity of the sequencing technology.

Aims: We have applied an integrated approach to determine the TP53/17p status in 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 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 referred to our diagnostic center for analysis of 17p deletion, 17p CNLOH and the TP53 mutation. To determine the TP53 mutation status (exons 2-11) sequence analysis was performed by next generation sequencing 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 (Affymetrix) platform which allows the detection of copy number alterations (e.g. deletions) as well as CNLOH.

Results: Twenty-one of the 179 CLL patients exhibited a loss or CNLOH of the short arm of chromosome 17 as demonstrated by oligo/SNP-based array. Eight of these cases had a CNLOH of 17p and would not have been observed in conventional metaphase FISH. In three cases FISH had been performed. In addition, by applying TP53 mutation analysis 26 patients were identified in whom the TP53 gene was inactivated. In six of these cases the mutant allele frequency was below 20% and would have escaped detection by Sanger sequencing. Therefore ten of the 26 (38%) patients with TP53/17p aberration would not have been indentified in cases where conventional FISH was performed and, therefore, the higher sensitivity of array sequencing can be inferred clonal evolution pattern for one pt.

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

Pairwise association showed statistically significant co-occurrence between tri(21) and mutations in KARS/BCTR (both q<0.05), NOTCH1 mutation and ZMYM3 (q=0.01)/SPEN (q=0.05) mutations, and TP53 mutation and del(17p) (q<0.01) complex karyotypes (q<0.05). When correlating with clinical response to lenalidomide, worse overall response (OR) in the untreated group was associated with del(17p) (p=0.019) and KRAS mutation (p=0.05), whereas as mutation in SF3B1 (p=0.026), MGA (del) (p=0.045), DXDX (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 with worse OR in lenalidomide-based therapies. TP53 and del(17p) were associated with worse PFS (p=0.015) in the untreated cohort.

To obtain comprehensive insights into the ontogeny and evolution of CLL subset #4 are characterized clinically by a young age at diagnosis and an indolent disease course, and molecularly by B-cell receptor Ig light chains. 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 rearrangement, the filtered reads were parameterized according to clonotypic barcode (Clusterves), usage of subset #4-specific V- and J-genes, CD3 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 processed sequences, which 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 targeted by the immune system, (ii) 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.
BACKGROUND: During normal hematopoiesis, a coordinated epigenetic and transcriptional programming is necessary to achieve lineage development. B cell differentiation is predominantly related to loss of DNA methylation at the enhancers and promoters of B cell-specific genes; e.g. transcription factors (TFs). In chronic lymphocytic leukemia (CLL), failure of proper epigenetic programming contributes to deregulation of B cell transcriptional programs and results in CLL phenotypes with highly variable outcomes. The mechanisms leading to failed epigenetic programming and to establishment of a CLL epigenome are not well understood. Genomic sites of failed epigenetic programming coincide with binding sites of key B cell TFs. Active DNA demethylation through TET-dioxygenase mediated conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and subsequent products is one of the mechanisms involved in physiological epigenetic B cell programming, and deregulation of this process could contribute to establishment of the CLL epigenome.

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

Methods: Clonal B cell specimens from 122 CLL patients were subjected to DNA methylation profiling using Illumina 450k arrays. 17 CCL and 4 healthy B cell samples (CD19+) were used for DNA methylation profiling using Illumina Epic arrays and for hydroxymethylated DNA immunoprecipitation (hMeDIP) using a monoclonal 5hmC mouse antibody and the NEBNext Ultra DNA Library Prep Kit for analysis on a Illumina Hiseq2000 sequencer. Global 5hmC levels were quantified by dot blots. TET2, and EB1F1 mRNA and protein expression was evaluated by qPCR and Western Blot, respectively.

Results: By dot blot, we found decreased 5hmC levels in CLL as compared to CD19+ B lymphocytes. 5hmC was further reduced in IGHV unmutated compared to IGHV mutated CLL patients. To identify distinct regions with gain or loss of 5hmC, we performed genome-wide 5hmC profiling by hMeDIP. We confirmed a significantly lower number of hydroxymethylated peaks in CLL (137114) compared to HBC (249421) which remained stable when separating the patients based on Richter's transformation (92915/293476) and with additional data sets (63667/249421). Differential binding analysis (DBA) revealed 5988 significantly differentially hydroxymethylated reads between CLL and HBC samples (FDR<0.05). Pathway analysis showed that regions which lost hydroxymethylation in CLL were involved in B cell receptor (BCR), Class I P38K, CXCR-4, c-Mec and IL3 signaling. To further identify mechanisms that are involved in failed hypomethylation and 5hmC loss in CLL, we aimed at profiling sequence characteristics at the respective genomic sites. In our genome-wide DNA methylation data set, we confirmed highly significant enrichment of the EB1F1 motif at the respective sites in 122 CLL patients. EB1F1 mRNA and protein expression was significantly reduced in the majority of 17 CLL samples compared to HBC. TET2, a potential interaction partner of EB1F1, was upregulated in CLL samples on RNA level and expressed to different degree on protein level.

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 EB1F1 and TET2 are mechanistically involved in insufficient hydroxymethylation and consequently failed DNA hypomethylation.

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Chronic lymphocytic leukemia and related disorders - Clinical

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

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 and eradication of MRD in some CLL patients. The IcICLLe Extension Study expands on the IcICLLe trial (ISRCTN12695354) 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 patients with CLL requiring treatment, 20 treatment-naïve (TN) and 20 relapsed/refractory (RR), to receive continuous ibrutinib therapy until confirmed MRD negative remission (<0.01% residual disease) or disease progression. The IcICLLe Extension Study adds 40 RR participants with CLL requiring treatment to receive continuous ibrutinib therapy from day 0 and 6 cycles of obinutuzumab from day 1. 30 participants have no prior ibrutinib treatment (ibrutinib-naïve), and 10 are pre-treated with ≥12 months of ibrutinib on IcICLLe. The primary outcome for the IcICLLe Extension Study is the proportion of patients achieving MRD-negative remission by IWCLL criteria (depletion of CLL below 0.01% in the peripheral blood and bone marrow) at or before 9 month assessment.

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

Results: 31 participants (22 ibrutinib-naïve and 9 pre-treated) are evaluable for response assessment after 1 month of combination treatment. There have been no reports of tumour lysis syndrome within the first month of combination treatment. There were 2 separate reports of grade 2 infusion related reactions, both on day 1 of obinutuzumab. In the 22 ibrutinib-naïve cases peripheral blood (PB) CLL counts remained at or below baseline levels in 17/22 cases from week 1 onwards. After 1 month of combination therapy the PB CLL count was a median 31% of baseline levels (range <1%-174%) compared to median 215% (range 29%-3570%) for RR patients on ibrutinib monotherapy. Percentage CLL cells in the bone marrow (BM) aspirate after 1 month of combination therapy reduced from a median 83% (range 23-94%) to a median 47% (range 5-85%) (P=0.003, Wilcoxon matched-pairs signed ranks). For RR patients on ibrutinib monotherapy there was no change in BM at 1 month; baseline median 85% (range 11-96%) compared to median 86% (range 50-98%), P=0.96. Changes in BM aspirate CLL percentage were confirmed by morphological assessment of a trephine biopsy with all evaluable patients receiving obinutuzumab showing improvements in the cellularity and/or extent of infiltration.

BM assessment at 1 month was not mandated for the 9 pre-treated patients but all showed decreased PB CLL counts with 4/9 achieving <0.01% residual disease within 3 months of starting obinutuzumab. 13 patients have completed 6 months of obinutuzumab treatment with marrow assessment at 9 months showing a further ≥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.

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CHRONIC LYMPHOCYCTIC LEUKEMIA PATIENTS EXPRESSING THE LIGHT CHAIN IGLV3-21 OR THE IGHV MUTATIONAL STATUS
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Background: The immunoglobulin heavy-chain gene (IgH) mutational status is currently considered the gold standard of prognostication in Chronic Lymphocytic Leukemia (CLL): unmutated (UM) immunoglobulin heavy chain region (IgH) is associated with poor prognosis while patients with mutated IgH (M) have more indolent disease. An exception are patients with IgHV3-21/IgLV3-21 who have poor prognosis irrespectively of the IgHV mutational status. Interestingly, IgH3-21 is co-expressed with IgLV3-21 in the majority of cases.

Aims: Here we aimed to study the impact of the light chain IgLV3-21 on CLL prognosis. This light chain has never been characterized independently of the heavy chain IgH3-21.

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 IgLV3-21 on treatment-free (TFS) and overall (OS) survival. IgLV3-21 positivity was determined by real-time PCR and confirmed by Sanger sequencing.

Results: Among the 32 patients with aggressive CLL, we found that 9 (28%) patients who had an IgLV3-21 rearrangement, but only 1 patient carried the heavy chain IgH3-21: IgLV3-21 patients had a median TFS of 17 months compared to 44 months in patients with another light chain (P=0.0270). Similarly, IgLV3-21 patients had a shorter median OS (88 months vs >192 months, P=0.0287). We verified these results in 2 independent cohort obtained at diagnosis. In cohort B (n=270), 30 (11%) expressed an IgLV3-21 light chain and 10 (4%) an IgH3-21 (of which 8/10 also carried the light chain IgLV3-21 rearrangement). Patients with IgLV3-21 had a median TFS/OS of 29/183 months compared to patients without IgLV3-21 who had a median TFS/OS of 88/292 months (P=0.0003/P=0.0142). In cohort C (n=103), 9 (9%) expressed an IgLV3-21 light chain but only 1 (1%) had a heavy chain IgH3-21. In this cohort, IgLV3-21 patients had a median TFS of 21 months not statistically different from IgH3-21 patients (28 months) while IgLV3-21 patients had a median TFS of 93 months (P=0.0001). We then pooled the 3 populations (n=405) in order to increase the under-represented subgroups and analyzed the association of the IgLV3-21 with the IgHV mutational status: patients with either IgH3-21 or IgLV3-21 (with a M or UM IgH) displayed a prognosis similar to UM patients: median TFS was 129, 48, 36, 24, 23 months for M, IgLV3-21/M (P=0.0005), UM (P<0.0001), IgLV3-21/UM (P=0.0001) and IgH3-21/UM (P=0.0001) patients, respectively (Figure 1A). Similar results were observed for OS with a median OS of 292, 88, 174, 90 and 183 months M, IgLV3-21/M (P=0.0001), UM (P<0.0001), IgLV3-21/UM (P<0.0001) and IgH3-21/UM (P=0.0021) patients, respectively (Figure 1B). If all IgLV3-21 (n=48) were considered independently of their heavy chain, IgLV3-21 median TFS (24 months) was similar to UM patients (36 months, P=0.5824) and statistically different from M patients (129 months – P<0.0001, Figure 1C). Similar results were observed for OS (Figure 1D).

Figure 1.

Summary/Conclusions: Our results highlight for the first time the independent prognostic significance of the light chain (IgN)-21 in CLL: the presence of an IgLV3-21 light chain confers a poor prognosis similar to UM patient irrespectively of concurrent expression of IgH3-21 heavy chain or IgHV mutational status.
DURABILITY OF RESPONSES ON CONTINUOUS THERAPY AND FOLLOWING DRUG CESSATION IN DEEP RESPONDERS WITH VENETOCLAX AND RITUXIMAB

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Background: Venetoclax is a potent BCL2-2 inhibitor that is approved as monotherapy for certain patients with relapsed or refractory chronic lymphocytic leukemia (CLL) in the United States, the European Union, and other countries.

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

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

Results: Forty-nine patients, with a median of 2 (range: 1–5) prior regimens, were enrolled. As of July 2016, the overall response rate was 86%, the CR rate was 51%, and the bone marrow MRD-negativity rate was 57% (28/49) [Seymour et al. Lancet Oncol 2017]. The 24-month estimate for progression free survival was 78.8% and that for duration of response was 87.8% (100% for patients with MRD-negative CR). Of the 28 patients attaining MRD-negativity, 22 achieved the status at 7 months, which was the first mandatory time point for assessment. The remaining six patients achieved MRD-negativity at the second assessment, which ranged from 12 to 22 months, since the timing of this test was not mandated. Twenty (41%) patients discontinued the study. Eleven had progressive disease while on therapy: five with Richter’s transformation between 1–9 months and six with CLL progression after a median of 26.4 months (range: 12–37). The other nine patients: withdrew consent (n=3), failed to report for follow-up evaluations (n=1), discontinued due to adverse events related to venetoclax (n=2; tumor lysis syndrome and worsening of peripheral neuropathy), or discontinued due to adverse events considered not related to therapy (n=3). Seventeen patients continued on therapy: 8 MRD-negative CR, 2 MRD-positive CR, 5 MRD-negative PR, and 2 MRD-positive PR. Median duration of response on therapy is 27.9 months (range: 20.3–40.2). Sixteen patients discontinued venetoclax and remained on study as allowed per protocol following the achievement of a deep response (12 MRD-negative CR, 2 MRD-negative PR, 2 MRD-positive CR) (Figure 1). Their median time on venetoclax is 16.3 months (range: 5–38). Twelve of these patients remain in active follow-up and four discontinued without evidence of progression after achieving MRD-negative CR. Two patients with MRD-positive CR had increasing absolute lymphocyte count (ALC) and asymptomatic progression 24 months after stopping venetoclax. Both re-started venetoclax, 2 and 6 months after ALC >5x10^9/L, and achieved partial remissions. The 10 patients with MRD-negativity in the bone marrow who remain in follow-up have a median duration of ongoing response off venetoclax of 13 months (range: 2–34).

Summary/Conclusions: Venetoclax with rituximab induces deep and durable responses, with 51% patients achieving CR and 57% achieving narrow 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.

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

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Background: Recurrent mutations in genes such as TP53, SF3B1 and NOTCH1 are frequent in CLL and have in previous studies been associated with outcome. SF3B1mut, TP53mut, BIRC3mut and XPO1mut were adverse prognostic factors in patient cohorts with different therapies, and NOTCH1mut associated with poor outcome when rituximab was added to standard chemotherapy. In the COMPLEMENT-2 trial FP+ofatumumab vs FC was associated with NOTCH1mut as a predictive factor in the context of chemotherapy.

Aims: We assessed the incidence and clinical associations of mutations in TP53, SF3B1, NOTCH1, ATM, BIRC3, FBXW7, MYD88, EGR2 and XPO1 in the COMPLEMENT-2 trial (relapsed/refractory CLL, FC vs FC+ofatumumab). Patients were enrolled between November 2013 and May 2015 (Robak et al., Leuk Lymphoma, 2017).

Methods: Baseline samples were available from 325 of 365 patients (89%) representative of the full analysis set of the clinical trial. Mutation analyses were performed via custom targeted Next Generation Sequencing (NGS) for TP53, ATM, BIRC3, FBXW7, MYD88, EGR2 and XPO1 in the COMPLEMENT-2 trial (relapsed/refractory CLL, FC vs FC+ofatumumab). 170 consecutive patients were included (Robak et al., Leuk Lymphoma, 2017).

Results: In total we identified 365 mutations across the 9 genes in 202 of 325 patients (62.2%), with incidences of SF3B1mut 19.7%, TP53mut 18.8%, NOTCH1mut 16.3%, ATMmut 13.8%, XPO1mut 11.4%, BIRC3mut 4%, EGR2mut 3.1%, FBXW7mut 2.7% and MYD88mut 0.9%. We identified a variety of associated mutational subgroups with genetic, clinical and laboratory parameters, such as TP53mut with del17p (p<0.01), NOTCH1mut, FBXW7mutand BIRC3mut with +1q2 (p=0.01, p=0.01 and p=0.05) and ATMmut with del11q (p=0.01). XPO1mutand ATMmut associated with unmutated IGHV, CD79B expression on cell surface measured via flow cytometry was lower in ATMmut patients, whereas CD20 expression did not differ among the different mutational subgroups. TP53mut, EGR2mutand SF3B1mut patients had worse overall response to therapy (86% p<0.01, 50% p=0.02 and 72% p=0.05 respectively, vs 81% overall). Similar to the full analysis set, FCO as compared to FC resulted in significant improved PFS (median 28.1 vs 18.8 months, HR=0.67, p=0.01), TP53mut and XPO1mutwere adverse prognostic factors for PFS (HR 1.93 p<0.01 and HR 1.85, p<0.01 respectively), but only TP53mut for decreased OS (HR 2.11 p<0.01). All other mutations, in particular SF3B1mut and NOTCH1mut, did not significantly impact PFS or OS. To identify factors of independent clinical

Figure 1.
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), del(17p) (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). Del(17p) (HR 4.79 p<0.01), unmutated IGHV (HR 1.69 p<0.04) and TP53mut (HR 1.76 p<0.03) were identified as independent prognostic factors for OS. With focus on the predictive value of gene mutations, we found a beneficial effect of the addition of ofatumumab to chemotherapy irrespective of TP53 mutation (HR 0.52 p=0.02 for TP53mut and HR 0.68, p=0.02 for TP53mt). Regarding NOTCH1, ofatumumab was only beneficial in NOTCH1mt but not in NOTCH1mt patients (HR 0.64, p<0.01 and HR 0.86, p=0.07) (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, a higher ofatumumab addition to FCO treatment was observed among NOTCH1mt but not among NOTCH1mt patients indicating NOTCH1 mt status as a predictive marker in the context of type-1 CD20 antibody addition to chemotherapy.

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

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

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

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

Results: Median pt age was 61 yrs (range 35–90); 68.6% were male; 44.1% had Rai stage 3–4. For evaluated pts, IGHV status was 32.9% mutated and 67.1% unmutated. Incidences of trisomy 12, normal cytogenetics, and deletions of 13q, 11q, and 17p were 23.4%, 37.5%, 17.2%, 15.6%, and 6.3%, respectively. Investigator-assessed CR rate was 49.0% (95% CI 39.9–59.1) and ORR was 89.2% (95% CI 81.5–94.5) after 6 cycles. MRD negativity in blood, as measured by next-generation sequencing, was achieved in 42.7% of pts at the end of induction response assessment and in 75.5% of pts at any time following treatment. MRD negativity in bone marrow (BM) was 60.8% in pts with BM samples. The most common adverse events (all grades [Gr]) were injection reactions (72.5%), nausea (52.0%), pyrexia (36.3%), neutropenia (34.3%), fatigue (34.3%), constipation (26.5%), and rash (25.5%). The most common Gr 3–4 adverse event was neutropenia (26.5%). Incidence of Gr 3–4 infections was 11.8%. Incidence of tumor lysis syndrome was 4.9% (all Gr 3). Three pts died; none were deemed related to study treatment or CLL by investigators.

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

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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 registration of 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 for pts who did not receive therapy were 23, 36 and 49% in 1989-2000, as compared with 17, 26 and 42% in 2001-2014. Overall, when corrected for age and sex, pts diagnosed in 2001-2014 had 49% lower excess mortality during the first 10 years after HCL diagnosis, as compared with pts diagnosed in 1989-2000 (P=0.005). Ten-year RS (95% confidence intervals) was impressive for pts age 18–59, namely 92% (88% - 96%) and 98% (94% - 100%; P=0.176) 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=0.009; Figure 1b), and for pts age ≥70 from 67% (49% - 86%) to 85% for 80% - 92%; P=0.36; Figure 1c) between the first and last periods. In addition, older age (P<0.001), but not sex (P=0.058), was associated with higher excess mortality.

Figure 1.

Summary/Conclusions: The incidence of HCL remained stable during a 26-year period in the Netherlands. RS for pts diagnosed in the period 2001-2014 eventually reached a plateau, indicating that by then their survival is comparable to that of the general population. Survival was already excellent for younger patients throughout the entire study period. Survival improvement was most pronounced for pts 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: CLL-IPI is a prognostication tool to stratify patients with chronic lymphocytic leukemia (CLL) for low, intermediate, high, or very high risk. CLL-IPI uses age, Binet stage, beta-2-microglobulin, 17p deletion / BR but not GCLB / RCLB / CLB. Median observation time was 55 months. All patients had CIRS score when estimating prognosis by CLL-IPI in CLL.

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

Results: Median age was 64 years; 69% of patients were males, and 50% had ECOC performance score of 1 or higher. Complete information on age, Binet stage, beta-2-microglobulin, 17p deletion / TP53 mutation, IGHV mutational status, but not comorbidity as weighted factors to model prognosis. CIRS is a tool which allows assessing and quantifying burden of comorbidity in individual patients.

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

Summary/Conclusions: Findings suggest that CIRS provides prognostic information in addition to the CLL-IPI and may be a useful tool for comorbidity assessment (e.g. by CIRS) in addition to the CLL-IPI therefore appears reasonable when estimating overall prognosis and deciding treatment in CLL.
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FINAL RESULTS OF THE PHASE IB/GALTON TRIAL IN CHRONIC LYMPHOCYTIC LEUKEMIA: DURABLE REMISSIONS WITH FRONTLINE OBINUTUZUMAB (G) PLUS FLUDARABINE/CYCLOPHOSPHAMIDE (G-FC) OR BENDAMUSTINE (G-B)


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

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

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

Results: 21 pts were enrolled in the G-FC arm and 20 in the G-B arm. Median age was 60 (25–80) years, 78% of pts were male, and around one-third had Rai stage III/IV disease. Median time from diagnosis to therapy was 24 mo (G-FC: 12, G-B: 36). 37 ptns were alive in follow-up: G-FC (n=18: 2 lost to follow-up) and G-B (n=19: 1 pt died 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 Gr5). Gr4 leukenemia/neutropenia, small cell lung cancer and Gr4 pneumoithorax, 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, 17/36 (47.3%) in the G-FC arm and 19/35 (54.3%) in the G-B arm.

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

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

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

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

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

Results: Between 2006 and 2013, 50 patients with a median age of 56 years old underwent RIC-SCT for the treatment of CLL. The median time from diagnosis to RIC-SCT was 4.7 (0.6-22.9) years. Fourteen (28%) patients had 17p deletion at time of transplantation. CLL-IPI prognostic score calculated prior to transplant was intermediate in 30%, high in 42% and very high in 28% of patients. Disease status at the time of transplant was partial or complete remis-
Chronic myeloid leukemia - Clinical 1

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

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Background: Several studies showed that tyrosine kinase inhibitors (TKIs) can safely be discontinued in patients with sustained deep molecular response. So far, deep molecular response (DMR) and treatment duration were predictive for successful treatment-free remission (TFR) whereas age, risk scores, gender and molecular response level before stopping were without influence (Mahon FX et al. British Journal of Hematology 2016; 163:737-746). In this substudy of the EUROSKI trial we aimed to investigate the impact of DMR expression on TFR. Methods: Expression levels of the influx transporters OCT1, ABCG2, and the efflux transporter ABCB1 were measured by qRT-PCR in the blood of patients, enrolled in the EUROSKI trial and screened in our center. Results: In our cohort, 132 chronic phase CML patients discontinued TKI treatment OCT1 and the efflux transporters ABCG2 and ABCB1 (MDR1) were quantified in order to investigate their impact on TFR. As all patients are in DMR, we investigated whether these transporters confer a constitutional disposition for TFR.
Aims: In a substudy of the EUROSKI trial, expression levels of the influx transporter OCT1 and the efflux transporters ABCG2 and ABCB1 (MDR1) have been quantified in order to investigate their impact on TFR. All patients are in DMR; we investigate whether these transporters confer a constitutional disposition for TFR.
Methods: The expression levels of OCT1, ABCG2 and ABCB1 have been determined by an absolute transcript quantification method in the peripheral blood of patients, enrolled in the EUROSKI trial and screened in our center. Minimal inclusion criteria were three years TKI treatment and one year MR4+5 duration (BCR-ABL<0.01%). Plasmid standards have been included using the genes OCT1, ABCG2, ABCB1 together with GUS as reference gene. Expression measurements were performed by qRT-PCR on baseline (day of stopping TKI treatment) samples. Cutoff levels were determined by the minimal p-value approach and adjusted for multiple testing by the Bonferroni method. The predictive significance of the influx and efflux channel transcript levels was determined by a multivariate Cox’s regression model. Relapse has been defined as loss of major molecular response at one time point.
Results: In our cohort, 132 chronic phase CML patients discontinued TKI treatment OCT1 (87% imatinib 1st line treatment), showing a relapse rate of 46%. Median MR4 and TKI treatment duration was 43.7 years respectively. The majority of patients were positive for the e14a2 transcript (e14a2+; 63%, e13a2+; 28%, e13a2+e14a2+; 9%). The mean expression of OCT1 and ABCB1 between ‘relapse’ and ‘no-relapse’ patients showed no significant difference (p=0.70 and 0.86 respectively). The expression of ABCG2 showed a weak differential expression (1.1% vs 0.8%, p=0.065). Cutoff analyses showed a significant risk stratification only for the ABCG2 efflux transporter at a distinct cutoff value of 4.5% (p=0.04). Patients with an ABCG2/GUS transcript level above 4.5% (n=93) had a 30-months TFR of 47%, whereas patients with low ABCG2 expression (<4.5%, n=39) had a 12-months TFR of 67%. The hazard ratio and predictive significance of the ABCG2 transcript levels were investigated by a multivariate Cox’s regression model. Only ABCG2 expression was retained as independent covariate in this model (p=0.033). Thus, patients with an ABCG2/GUS transcript level above 4.5% showed an up to two-time higher risk of relapse after treatment discontinuation (HR=2.1, 95% CI: 1.06-4.06). 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.

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HLA-G MOLECULES AND CLINICAL OUTCOME IN CHRONIC MYELOID LEUKEMIA

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Background: The human leukocyte antigen-G (HLA-G) gene encodes a tolerogenic protein known to promote tumor immune-escape mechanisms.

Methods: The entire HLA-G gene was amplified by long-range PCR and sequenced using next-generation sequencing (NGS) with Illumina’s Nextera® technology and a 300 bp paired-end read protocol. The BioVendor sHLA-G ELISA (RD194070100R sHLA-G ELISA - EXBIO Praha a.s. BioVendor) immunoassay was used for the quantitative measurement of HLA-G1 and HLA-G5 soluble forms in EDTA-plasma samples.

Results: The frequency of the G*01:03 allele was significantly associated to G01:03:02 allele had a significantly higher mean value of soluble HLA-G compared to patients carrying G*01:01:03 (109.2±39.5 vs 93.9±8.8 units/ml; p=0.03), and showed significantly lower EFS compared to patients with other allelic combinations (62.3% vs 90.0%; p=0.05). Moreover patients carrying the G*01:01:03 allele had significantly higher rates of MR4+5 (100% vs 85%), with earlier achievement of deep MR4+5 (median of 8 vs 58 months, p=0.001). TKIs were discontinued in 24 patients after 2 years of confirmed MR4+5. Treatment free remission (TFR) was 57.7%. None of the patients homozygous for the G*01:01:01 or G*01:01:02 allele remained in TFR (0% vs 68.4%, p=0.023) (Figure 1). All patients carrying the G*01:01:03 allele remained in TFR.

Figure 1.

Summary/Conclusions: HLA-G alleles with higher secretion of soluble HLA-

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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.

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

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

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

Methods: Differentiating 3T3-F442A mouse adipocytes were incubated with clinically relevant concentrations of NILO (1-20µM) and IMA (5µM), in the presence or absence of telmisartan (1-10µM), an angiotensin receptor blocker with potential beneficial effects on insulin sensitivity and lipid homeostasis. Cytoxicity and adipogenesis were assessed by MTT assay and Oil Red O staining, respectively. Expression of adipogenic gene, adiponectin, corresponding receptor gamma (PPARγ), Lpin1 (Lpin1), sterol regulatory element-binding protein 1 (Srebp1) and glucose transporter 4 (Glut4) were investigated by quantitative PCR and secreted adiponectin was measured by ELISA. Plasma samples were collected from 30 CML patients on either NILO (first line, n=6; second line, n=9) or IMA (first line, n=15) at baseline and at 3 and 12 months of therapy, and adiponectin was measured by ELISA. Data are presented as mean ± SD for 20µM incubations but full concentration response relationships were measured.

Results: Neither NILO nor IMA were cytotoxic to the adipocytes at clinically relevant concentrations. A dose dependent reduction in lipid accumulation was observed for NILO (for 20µM, 0.76 ± 0.055 absorbance units; p<0.01) but not IMA (0.98±0.007), compared to vehicle control. NILO, but not IMA, dose dependently downregulated the mRNA expression of PPARγ (52% downregulation), Lpin1 (28% downregulation) and Srebp1 (54% downregulation). Both NILO and IMA resulted in significant downregulation of GLUT4 mRNA (NILO, 93%; IMA, 79%; p<0.01) and of secreted adiponectin (NILO, 5.99ng/ml; IMA, 31ng/ml; both p<0.01 in comparison to vehicle control, 79.2ng/ml). Co-incubation with telmisartan resulted in significant reversal of NILO-mediated effects on lipid accumulation, adipogenic gene expression and adiponectin secretion. In the patient cohort, IMA resulted in a significant increase in adiponectin levels at 3 (38.4±7mg/l; p<0.01) and 12 (36.7±2.7mg/l; p<0.01) month time points compared to baseline (27.3±5.7mg/l). In contrast, second line NILO showed a trend for reduction in adiponectin at both 3 (15.2±1.8mg/l) and 12 months.

Results demonstrate the durability of TFR after stopping NIL in pts who achieved a sustained deep MR after switching from IM to NIL.
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**EARLY PREDICTION OF THE MOLECULAR RESPONSE TO BCR-ABL1 TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA**


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**Background:** A BCR-ABL1 transcript level at 3 months after the initiation of imatinib therapy has shown to predict the long-term clinical outcomes in 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 (MRM) achievement after 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, 6, and 12 months after the initiation of a TKI. A validated qPCR assay was tested on a certified CAP MR4.7 sample.

**Results:** 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 0.87. 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 – t/40]^t/8. Where MR0 is the predicted molecular response at baseline; t, t, time post TKI therapy; γ, slope factor; ββ, time required to achieve 50% reduction in MR. Statistical analysis showed significant differences 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 β (0.052 vs 1.12 month; p=0.05).

**Summary/Conclusions:** We report here on an optimized digital PCR assay with a high specificity and sensitivity for the detection of BCR-ABL1 transcripts at MR4.7. This assay allows accurate detection of MRD in BCR-ABL1 positive diseases with a detection rate of 100% for MRS and 67% for MR5.5 in a 4 wells analysis.

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**VALIDATION OF THE EUTOS LONG TERM SURVIVAL SCORE IN DUTCH CML-PATIENTS**

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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 used 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 chance of achieving CCyR at 18 months, as a proxy for survival. However, since the major causes of death of CML patients are no longer CML-related, the need for baseline risk prediction has shifted from overall survival towards disease specific mortality. Therefore, recently the EUTOS long-term survival (ELTS) score was introduced to predict the risk profile of 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=0.022) 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.
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ACUTE MYELOID LEUKEMIA ALTERS THE PERMEABILITY OF THE BONE MARROW VASCULAR MICROENVIRONMENT, FOSTERING DISEASE PROGRESSION AND DRUG RESISTANCE

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Summary/Conclusions:

Figure 1. ACUTE MYELOID LEUKEMIA ALTERS THE PERMEABILITY OF THE BONE MARROW VASCULAR MICROENVIRONMENT, FOSTERING DISEASE PROGRESSION AND DRUG RESISTANCE

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 vascular permeability. Moreover, vascular permeability was increased as measured via two-photon imaging. Interestingly, induction chemotherapy failed to normalize the altered vascular permeability in the BM, although it significantly reduced the AML engraftment. Via high-throughput transcriptomic analysis, we showed that AML-induced hypoxic environment altered the molecular signature of vascular endothelial cells, activating pro-angiogenic pathways and positively regulating the response to hypoxia. We identified increased nitric oxide (NO) as the major mediator of the AML-induced vascular leakiness in the BM. Notably, increased NO levels were found also in BM aspirates of patients at diagnosis compared to healthy donors, and failure in reducing NO levels after chemotherapy applied in vivo was associated with an increased number of endothelial cells.

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).

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BUILDING HUMAN BONE MARROW-LIKE MODELS TO STUDY NICHE INTERACTIONS

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Background: Previously, we have reported that our human bone marrow-like scaffold (huBM-sc) xenograft model allows the engraftment and outgrowth of normal and malignant hematopoiesis (e.g. multiple myeloma (MM) and acute lymphoblastic leukemia (ALL)) (Groen et al. Blood 2012; Gutierrez et al. JCI 2014) and more recently acute myeloid leukemia (AML; Antontelli 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 osteoestals 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 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 in scaffolds we performed: i) scaffold material composition (biomechanics to physiological; TCP vs tricalcium phosphate (TCP)); ii) scaffold shape (particles vs tubes); iii) different types of matrix for cord blood-derived endothelial progenitor cells (CB-EPCs) embedding.

Results: Histological analysis of these fully humanized scaffolds showed a large hematopoietic potential from hCD34+ cells in the huBM-sc model 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 MM 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.

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


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

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

Figure 1.
TEMPLETED 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 identified in B-cell repertoires of healthy donors and whether such insertions could be functionally explored to explore their biological function.

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

Results: Six unique VDJ sequences, all from the same donor, carried a templated insertion in-frame (E=10–4 – 6). 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 sequencese positioned close together and in close proximity (<25µm) to the extraluminal surface of sinusoidal endothelium. The IgA VDJ carrying the LAIR1 templated insertion produced no detectable IgA, IgG, and IgE isoforms 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.

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

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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-10 double knockout mice, which were treated with bleomycin to induce pulmonary fibrosis.

Results: Caspase activation is involved in the generation of M2 polarized macrophages. Caspase inhibition delays the ex vivo differentiation of peripheral blood monocytes exposed to CSF1 and modifies the phenotype of generated macrophages, e.g. cell shape, surface markers and cytokine secretion. In mice, caspase knock-out also modified the phenotype of monocytes induced to differentiate into macrophages. Caspase activation appeared to be prominent at the mitochondria level and responsible for the NOX2-dependent generation of cytosolic radical oxygen species (ROS). Activation of the NOX2 complex is associated with p47phox cleavage by caspases. Mice treated with bleomycin typically develop a pulmonary fibrosis. Bleomycin-induced lung fibrosis was delayed in monocyte-restricted caspase-8 knockout mice and prevented by treatment with a caspase 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 inhibitor molecules may be an exciting therapeutical strategy to modulate macrophage polarization with diverse applications including cancer treatment.
clastogenic assays were used to elucidate the down-stream effects of the elevated 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 in BM, decreased bone disease, and lower expression of OC-associated genes. Finally, the presence of CXCL13 in primary MM samples was evaluated. B-cell 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).

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RE-ORDERING THE B CELL DEVELOPMENT HIERARCHY IN HUMAN FETAL BONE MARROW: CHARACTERISATION OF A NOVEL HUMAN FETAL B PROGENITOR
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Methods: Here, we employed the Confetti allele, in which a cassette targeted to the ROSA26 locus randomly and permanently marks cellular progeny with GFP, YFP, RFP or CFP when exposed to Cre recombinase. We determined empirically that sample-to-sample variance in the distribution of Confetti colors in the blood of the following adult mice inversely correlated with the number of hematopoietic precursors specified during the indicated window of embryonic development.

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 a potential novel target for the diagnosis and treatment of MM.

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HUNDREDS OF EMBRYONIC HEMATOPOIETIC PRECURSORS CONTRIBUTE TO LIFE-LONG HEMATOPOIESIS
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Background: Prior studies of the frequency of emerging hematopoietic stem cells (HSCs) and their precursors during mammalian ontogeny have all required ex vivo transplantation, transgenic reporter assays, or transplantation with few HSCs from fetal BM.

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

Methods: Here, we employed the Cr<sup>+</sup> allele, in which a cassette targeted to the ROSA26 locus randomly and permanently marks cellular progeny with GFP, YFP, RFP or CFP when exposed to Cre recombinase. We determined empirically that sample-to-sample variance in the distribution of Confetti colors in the blood of the following adult mice inversely correlated with the number of hematopoietic precursors specified during the indicated window of embryonic development.

Results: An inverse correlation of 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 embryonic development.

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


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Background: Maintaining immune tolerance requires the production of Foxp3+ regulatory T cells (Treg) in the thymus. Activation of NF-kB transcription factors is critically required for Treg cell development, partly via initiating Foxp3 expression. NF-kB 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-kB regulator A20 in Treg cell development and function.

Methods: We used A20F/F 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 bone marrow in vivo to analyze whether one bone marrow compartment would outperform another or would favor development of certain T cell or other immune cell subsets. We performed allogeneic hematopoietic stem cell transplantation with WT BM+T cells vs WT vs A20-deficient Treg cells to analyze whether A20-deficient Treg cells would reduce GVHD to the same extent as WT Treg cells.

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

Summary/Conclusions: In light of the largely anti-inflammatory effects that have been attributed to A20 in many cell types, this proinflammatory aspect of A20 physiology in effector and regulatory CD4+ T cells is particularly important since it may contribute to a change of perception of the functions of A20 as a negative regulator of NF-kB 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.

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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 immunological disorders, and therefore it is critical to fully understand the transcriptional network that controls their generation and activity. Differentiation of progenitors to mature mast cells is promoted by several transcription factors, such as GATA1, GATA2, STAT5, and MITF, and requires downregulation of C/EBPα. Recently, we identified another member of the C/EBP family of transcription factors, C/EBPγ, as a direct C/EBPα target gene. However, the role of C/EBPγ in mast cell development 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 Cebpγ conditional knockout mice, which allow excision of Cebpg in the hematopoietic system from the early embryogenesis. We employed Cebpgflox/flox Vav-1Cre- and Cebpgflox/flox Vav-1Cre+ mice, referred here as WT and CebpgKO, 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). Furthermore, 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 vitro, 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. Depletion potential of BMMCs was specified by measuring the percentage of b-glucuronidase released to the supernatant upon anti-TNP IgG 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 controls. Functionally, we demonstrated that deletion of Cebpg reduced mast cell migration towards antigen, SCF or PGE, and impaired degranulation upon FcγR-mediated activation. Further, BMMCs exhibit increased expression of C/EBPα in the absence of CEBPγ.

Summary/Conclusions: In summary, we revealed CEBPγ 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.

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TRANSCRIPTIONAL DIVERSITY AND DEVELOPMENTAL POTENTIAL OF EARLY HEMATOPOIETIC PROGENITORS REVEALED BY CELLULAR BARCODING AND TRANSCRIPTOME-WIDE PROFILING
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Background: One of the main complications of anti-cancer therapies or bone marrow transplantation protocols is their deleterious effect on the blood system, leading to prolonged neutropenia and increased risk for infections. Manipulating hematopoietic stem cells differentiation pathways to favor production of specific lineage-committed progenitors might optimize blood recovery.

Aims: In this study we aimed 1) to determine and quantify the contribution of medullary progenitor populations (MPP) to the repopulation of the T cell pathway using the barcode cellular labelling strategy that we have previously developed and 2) to decipher the heterogeneity of these MPP at the transcriptional level.

Methods: Three different MPP subsets, of the following phenotype: VCAM1+Flt3 (MPP1); VCAM1-Flt3+ (MPP2) and VCAM-1Flt3+ILR7 (CLP), were tagged with different barcodes carried by a collection of lentivirus and were analyzed by flow cytometry. For whole transcriptome-strand-specific sequencing, three biological replicates, per cell population, were sequenced at high depth of coverage (2 x 120 million reads).

Results: The results allowed the in vivo dynamic tracking of the progeny of the barcoded progenitors in transplanted recipients. Moreover, transcriptome-wide profiling allowed to identify, by cluster analysis of RNAseq profiles together with gene ontology annotation, unique co-expressed markers for the prospective isolation of these populations. Unsupervised classification correctly classified reference surface markers, currently used to purify progenitors, which validate our bioinformatic methodology. Transcriptional regulation of these cell surface markers was further assess by searching for co-expressed transcription factors and enriched binding sites in their promoters. Their grouping enabled to establish undescribed regulatory networks, specific to each progenitor cell.

Summary/Conclusions: Collectively, the cellular barcoding tool and the molecular changes observed at RNA and functional levels as they occur in vivo in the context of physiologic commitment processes, highlighted data that contribute to a deeper understanding of the dynamic of T-lineage differentiation and the lineage restriction process.
Hodgkin lymphoma

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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: HD9, HD12, HD15) were included.

Results: Among the 471 NLPHL patients, the median age was 39 years; 76% of patients were male; 53% of patients had early favorable, 16% had early unfavorable and 31% had advanced-stage disease. Study treatment consisted of ABVD- or BEACOPP-based chemotherapy alone, radiotherapy (RT) alone or combined-modality treatment (CMT). After a median observation of 9.2 years, the 8-year progression-free survival (PFS) rate for the whole patient group was 81.3% (83.2% for early favorable, 85.2% for early unfavorable, 76.2 for advanced stages). 80 of 471 patients (17%) had refractory disease or relapsed during the course of follow-up (primary disease progression: 76 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 disease-free survival was 95.1% for early favorable, 98.6% for early unfavorable, 87.4% for advanced stages). A total of 43 deaths were observed during follow-up resulting in a death rate of 1%. However, only a minority of these deaths was NLPHL-related (n=10). In contrast, most patients died from second malignancies (n=20) or due to other causes (n=13) such as heart failure and lung disease.

Summary/Conclusions: Taken together, the results from this large analysis on NLPHL patients prospectively treated and followed within randomized clinical studies for newly diagnosed HL indicate an excellent lymphoma-specific outcome. Nonetheless, further treatment optimization is necessary as the majority of deaths in NLPHL were related to second malignancies or other treatment-related late effects. Thus, future clinical trials including NLPHL patients should evaluate whether it is possible to reduce the treatment intensity without compromising efficacy. This goal may be achieved by the partial replacement of conventional chemotherapy by targeted drugs such as anti-CD20 antibodies as well as the reduction of RT fields and doses.

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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 J. Russell1,*, A. Collins2, A. Fowler1, M. Karanth4, C. Saha2, V. Shyamsundar1, J. Padayatty6, A. Hodson1, J. Wimperis2, S. Sadullah5, N. Grigoropoulos1, B. Uttenthal1, G. Follows1
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Background: The majority of young patients with advanced-stage Hodgkin lymphoma (HL) in the UK are managed with ABVD. However, following publication of the HD9 trial results in 2009, escalated-BEACOPP (escB) was introduced by some UK centres to improve disease control in poor-risk patients.

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

Methods: We collected data retrospectively from 8 hospitals in the East of England Cancer Network covering 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 for poor-risk patients, as determined by physician and patient choice; 44 patients were treated with escB, 202 with ABVD, 3 with alternative regimens. The first-line treatment of HL patients treated with escB compared with ABVD (5-year PFS 95% vs 80%; 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 86%; HR 2.64 (95% CI 1.43–24.89), p=0.012; 5-year OS 84%; p=0.0325). Twenty-nine ABVD patients and 3 escB patients had at least 1 subsequent stem cell transplant (including 6 allografts post-ABVD and 3 allografts post-escB), and there was equal use of consolidation radiotherapy between regimens (11% of both ABVD and escB patients). Treatment-related toxicity was not 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 aged ≥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.

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IMPACT ON SURVIVAL OF EARLY DETECTION OF RECURRENCE IN THE FOLLOW-UP OF HIGH RISK HODGKIN LYMPHOMA IN FIRST COMPLETE REMISSION E. D’Andrea1,*, M. Barba1, E. Consolo1, E. Carpinelli2, M. Di Martino1, N. Pugliese1, L. Simeone1, R. Dellà Pepe1, C. Giordano1, I. Cappuccio1, I. Zacheo1, G. Campagna1, C. Cerchione1, P. Zeppà2, C. Salvatore3, F. Pane1, M. Picardi1
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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/CT performed before ACR 2015). The second end-point was to assess if patients who received a conventional follow-up program including symptom assessment, blood tests and physical examination; in these patients imaging procedures were performed only in case of suspected relapse (Historical group). Subsequent patients (n=150) received routine imaging procedures comprising ultrasound (US) and chest X-ray (CXR) on admission, abdominal, and pelvic lymph nodes (SMAP US), and chest radiography (CXR), as integrated part of the follow-up strategy (Imaging group). Follow-up procedures were performed at 4, 8, 12, 16, 20, 24, 30, 36, 48, 60, 84, and 108
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 SMAP-US plus CXR to monitor patients with advanced stage HL. We show that SMAP-US plus CXR is a valuable tool to improve follow-up in patients with a high risk of recurrence. Our data indicate that the early detection of HL recurrence allows to begin rescue therapy in patients with a more limited disease and, consequently, increase its effectiveness in terms of probability to response and DFS.

P278
LATER LINE DRUG TREATMENT PATTERNS OF CLASSICAL HODGKIN’S LYMPHOMA PATIENTS IN CANADA, FRANCE, GERMANY AND THE UNITED KINGDOM
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Aims: To understand the drug treatment patterns of cHL patients in 3rd or later lines.

Methods: Real-world data were collected through a cross-sectional survey administered to physicians in Canada (Ca), France (Fr), Germany (Ge), and the UK between May and Sep 2016. Physicians provided data on the last 8 cHL patients receiving 3rd or 4th line drug treatment. Data captured included demographics, disease history and treatment patterns. Auto/allo stem cell transplants (auto/alloSCT) were not classified as a treatment line and limited data was available to determine when a SCT was received. Summary statistics were reported and differences between sub-groups assessed using chi-square tests.

Results: In total 116 physicians (Ca, 16; Fr, 31; Ge, 44; and UK, 25) provided information on 959 cHL patients (Ca, 128; Fr, 243; Ge, 351; and UK, 237) on 3rd or later lines of drug treatment. Data for 954 cHL patients on 3rd line drug treatment was captured. Patients had a mean age of 54.0 years (SD: 16.79) at the point of data capture. 57% were male, 43% female. 30% had bulky disease. 84% of patients had been tested for the Epstein Barr virus (EBV), 36% confirmed. The most commonly prescribed 3rd line drug treatment was a brentuximab-vedotin (BV) based regimen (35%). BV use was significantly different across the markets; Canada (34%), France (35%), Germany (30%) and the UK (44%) (p=0.010). The next most commonly prescribed 3rd line treatments were DHAP (8%), BEAM (7%) and bendamustine (7%). 4% of 3rd line patients received a PD-1 inhibitor. Of 3rd line BV patients the majority received ABVD (69%) or BEACOPP (19%) at 1st line. Their most common 2nd line drug treatments were DHAP (21%), ICE (10%), ESHAP (9%) and BEACOPP (9%). 59% of all 3rd line BV patients had undergone an auto/alloSCT at some point during their treatment history. Of 3rd line patients receiving non BV-based regimens 6% had been treated with BV previously (1st/2nd line). Of 3rd line patients treated with a PD-1 inhibitor 7% had been previously treated with BV. Data for 453 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, 36% confirmed. 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<0.001). At 3rd line this cohort had most commonly received DHAP (16%), BEAM (15%) or ICE (11%). 5% of 4th line BV patients also received a BV based regimen at 3rd line and 12% of 4th line patients had received a BV regimen at 3rd line. At 4th line 38% of this cohort received a PD-1 inhibitor, 19% bendamustine and 9% gemcitabine.

Summary/Conclusions: Real-world data indicates an unmet medical need for cHL patients with multiple relapses, reinforced by the use of PD-1 inhibitors in those relapsing post BV based regimens at 3rd line. There also appears to be no clear standard of care at 3rd line, again highlighted by use of a range of regimens and PD-1’s. This study was sponsored by Bristol-Myers Squibb.

P279
CHEMOTHERAPY AND RADIATION IMPROVE SURVIVAL IN EARLY STAGE CLASSICAL HODGKIN LYMPHOMA, A STATEWIDE CANCER REGISTRY ANALYSIS
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Background: Early stage classical Hodgkin Lymphoma (cHL) has been shown to have an excellent outcome. Recent studies have therefore focused on decreasing the toxicity that results from the addition of radiation therapy to chemotherapy. However, it remains unclear whether omitting radiation as part of the initial therapy of cHL is associated with a similar survival

Aims: The primary aim of this study is thus to investigate the outcomes observed in a statewide cancer registry for cHL patients treated with chemotherapy alone vs patients treated with both chemotherapy and radiation.

Methods: All adult patients (older than 18) diagnosed with cHL in Kentucky Cancer Registry (KCR) from 2005-2014 were retrospectively reviewed. Base-line characteristics including age at diagnosis, gender, histology, stage, B symptoms, extranodal involvement, and the site involved were collected. First line treatment modalities as well as overall survival outcomes were reviewed. Stage I and II patients without B symptoms were considered favorable, while those with B symptoms were considered unfavorable. Patients with stage III and IV disease were given an advanced stage designation. To adjust for selection bias, patient deaths during the first 6 months of diagnosis were censored for overall survival analysis.

Results: A total of 961 patients were identified. Median age was 41 (range 18-91) and 60.9% (n=585) were younger than 50. The group included a mild predominance of males (55.5%). Only 1.7% (n=16) had extranodal involvement at presentation. Of those with known histology (78.6%), the most common was nodular sclerosis (71.2%), followed by mixed cellularity (22.8%), lymphocyte rich (3.8%) and lymphocyte depleted (1.9%). Median follow up time was 45 months (range 0-136). The 10-year overall survival for the favorable group (n=329) was 77% (95% CI: 71.1-88.8) versus 68% for the unfavorable group (n=144) and 42% for the advanced group (372) (p<0.001). There was no statistical difference in survival between stage I (n=170), and stage II (n=385) disease (p=0.99). Treatment modalities were then compared for the favorable risk group alone. Those who received chemotherapy alone (n=145) were compared to those who received combined chemotherapy and radiation (n=148) as their primary therapy. The 10-year overall survival for the cohort receiving chemotherapy and radiation was 87% compared to 75% for those receiving only chemotherapy (p<0.001) (Figure 1). When adjusted by multivariate analysis for risk factors affecting 10 year survival of the favorable cohort, only age <50 and the treatment modality were independently associated with a statistically significant difference in overall survival (HR of 0.11 (p<0.001) and 3.94 (p=0.001), respectively).

Figure 1.

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

Figure 1.
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. Cohort 1 consisted of 178 patients who relapsed before January/2013, while Cohort 2 consisted of 36 patients relapsed after BV became available. Patient’s characteristics are shown in Table 1.

Table 1. Patients characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44 (16-81)</td>
<td>45 (19-81)</td>
<td>ns</td>
</tr>
<tr>
<td>Sex</td>
<td>97/81</td>
<td>20/16</td>
<td>ns</td>
</tr>
<tr>
<td>B-symptoms, (yes vs no)</td>
<td>135/43</td>
<td>21/15</td>
<td>ns</td>
</tr>
<tr>
<td>Stage (I-II vs III-IV)</td>
<td>94/80</td>
<td>23/13</td>
<td>ns</td>
</tr>
<tr>
<td>ECOG 0 vs 1 vs 2 vs 3</td>
<td>21/30/34/29</td>
<td>3/12/26/9</td>
<td>ns</td>
</tr>
<tr>
<td>Time from first SCT to relapse (months)</td>
<td>12.6 (9.7-15.5)</td>
<td>12.0 (9.7-15.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Time from first TC to relapse (months)</td>
<td>15.8 (9.7-15.5)</td>
<td>15.8 (9.7-15.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Time from first TC to relapse (months)</td>
<td>15.8 (9.7-15.5)</td>
<td>15.8 (9.7-15.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Initial therapy</td>
<td>56/30</td>
<td>10/6</td>
<td>ns</td>
</tr>
<tr>
<td>Dose intensity (90% vs 70%)</td>
<td>15/15</td>
<td>3/3</td>
<td>ns</td>
</tr>
<tr>
<td>BV vs 1st line therapy</td>
<td>38/18</td>
<td>2/0</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>BV vs 1st line after relapse</td>
<td>4/2 (25%)</td>
<td>0/0 (0%)</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

The following variables were included in a multivariate Cox proportional hazard regression analysis model: 1) age of patient, 2) Sex, 3) B-symptoms (yes vs no), 4) Stage of disease (I-II vs III-IV), 5) extranodal disease, 6) time from auto-SCT to relapse (≥12 vs >12 months), 7) Relapse before or after BV availability (Cohort 1 vs Cohort 2). In order to exclude any confounding effect of subsequent treatments, analysis was performed by censoring patients at the time of alloegenic SCT or treatment with immune checkpoint inhibitors (IC-ihibitors).

Results: In multivariate analysis the following variables were statistically associated with OS: 1) The presence of B-symptoms [HR=2.07, (95% CI, 1.39-3.07), p<0.001] and 2) Relapse in less than 12 months after auto-SCT [HR=4.09, (95% CI, 3.35-4.95), 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).

Summary/Conclusions: Patients in Cohort 2 survived longer even when censored for allo-SCT or treatment with IC-inhibitors. All patients in Cohort 2 treated with BV while only 18% of patients in Cohort 1 received treatment with BV. The results of our study strongly suggest that BV improves OS in patients with HL relapsed after auto-SCT. To our knowledge this is the first study showing an OS advantage of treatment with BV.

Figure 1.
tion was defined as imaging at or before week 12 of treatment, whereas late radiological evaluation was performed at or after week 16. Response evaluation was performed according to the Lugano Classification and its update regarding immunomodulatory therapy.

**Results:** Between 06/2015-11/2016, 87 patients were enrolled in a name-based program in Turkey. Two, 19, and 3 patients who had not yet received nivolumab, had not reach the time for early radiological evaluation, and who died before any radiological evaluation were excluded from the analysis. Thus, 63 patients from 23 centers were retrospectively analyzed. Median follow-up was 6 months, median age was 29 (18-75) and patients had a median 5 (2-11) previous lines of therapy. 44 patients (70%) had been treated by stem cell transplantation (SCT) and 48 (76%) patients had been treated by BV. The ORR was 68% with 15 CR (95%CI 0.020-0.28; CR 26%, PR 42%, SD 12%, PD 20%) among 59 patients evaluated in 12 weeks of nivolumab treatment. The ORR was 67% with 9 (24%) patients with CR after 16 weeks of treatment (95%CI 0.004-0.26; CR 24%, PR 43%, SD 6%, PD 27%). Estimated OS was 95% (95%CI 0.85-0.98) and estimated PFS was 71% (95%CI 0.55-0.82) at 12-months. Median OS was not reached, while, according to the late response rates, the median PFS was 14 months. However, it was only 3 months in patients with PD at the late radiological evaluation. Regarding responses to last treatment prior to nivolumab, we detected that 28 (67%) of 42 PD cases had objective early responses and 70% of PD cases had ORR in the late response evaluation (CR in 4, PR in 12 pts). 8 patients underwent transplantation following nivolumab. Among 5 patients who had been treated by allo-SCT, 4 had CR at the time of transplantation and they are alive with ongoing response. Safety profile was acceptable and only two patients required cessation of nivolumab due to serious adverse events: one due to autoimmune encephalitis and one due to aggravation of graft versus host disease. At the time of analysis, 40 cases were still on nivolumab treatment (64%). Among the 40 cases with early objective responses to nivolumab, 35 (88%) showed ongoing objective responses. All 24 cases with objective responses in the late evaluation had ongoing responses at the time of analysis (Figure 1).

**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 (Illumina). The sequencing was tailored to obtain a depth of coverage >2000x in >80% of the target region in all samples, which allowed a sensitivity of 3x10⁻³. The somatic function of VarScan2 was used to call non-synonymous somatic mutations, and a stringent bioinformatic pipeline was applied to suppress the background noise and to filter out sequencing errors.

**Results:** In newly diagnosed chL, genotyping of plasma cfDNA identified non-synonymous somatic mutations in STAT6 (43%), TNFAIP3 (43%), ITPKB (32%) BMI (21%), GNA13 (14%), CIITA (7%), XPO1 (7%) and CD58 (4%) among the most recurrently affected genes (Figure 1A-B). In refractory chL patients, genotyping of plasma cfDNA identified non-synonymous somatic mutations in ITPKB (44%), TNFAIP3 (33%), KMT2D (33%), BMI (33%), GNA13 (33%), XPO1 (22%), TE2 (22%), IKBKB (22%), BIRC3 (22%) and STAT6 (22%) among the most recurrently affected genes. Mutations of KMT2D (33%) and TE2 (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 cfDNA 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, CCR7 signaling, NF-kb 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.

**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.
Iron metabolism, deficiency and overload

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-treatment (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 value within this region was normalized for the corresponding blood pool index measured in the inferior vena cava to obtain LV-SUV.

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).

Here we aimed at evaluating the clinical relevance of these findings and in particular the parameters of vascular changes correlated 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: β-thalassemia patients show 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 show high systemic heme and iron levels, 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 parameters 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.

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ELEVATED SYSTEMIC HEME AND IRON LEVELS AS RISK FACTORS FOR VASCULAR DYSFUNCTION AND ATHEROSCLEROSIS: EVIDENCE FROM β-TALASSEMIA AND HEMOCHROMATOSIS 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 these mice.

Aims: For these reasons, 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: 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 Datastore (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 FCT claims (1st claim=index date), ≥2 DFX DT claims (last DFX DT claim ≥36 months of continuous activity in a 12-month period) pre-index. Medication possession ratio (MPR) (percentage of time with access to medication) was calculated for DFX DT during the “DFX DT period” (from earliest DFX DT claim to index date) and for DFX FCT during the “DFX FCT period” (from index date to end of data availability/ICT switch). Proportion of days covered (PDC) and persistence (without a gap ≥30 or 60 days between claims) were assessed in the 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 the Wilcoxon rank-sign 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 FCT vs DFX DT 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 FCT vs DFX DT was 0.83 vs 0.71 (p<0.001); 50.0% pts met the 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 FCT vs DFX DT (without a gap ≥30 days) was 87.2% vs 63.4% (p<0.01). Similarly consistent and significant results for PDC and persistence were observed using a 6-month time interval and/or a 60-day gap between claims.

Summary/Conclusions: Adherence and persistence to ICT was significantly improved in pts who switched from 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 low switching rate 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.

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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 method is well established, 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 T2-MRI method in a routine clinical practice setting.

Methods: A retrospective pre-post cohort study was conducted in pts switching between T2-MRI and SDPA T2-MRI LIC measurements. The geometric mean ratio of T2*/R2* LIC to SDPA T2-MRI LIC was 0.44 (95% CI 0.36 –0.55) indicating severe underestimation of LIC by the T2*/R2* method. The geometric mean ratios of the two LIC measurements were significantly different for the two scanners (0.28 for Philips and 0.68 for Siemens, p <0.0001) indicating that the bias of the T2*/R2* method against the reference standard is not universal but is dependent on both/either scanner type and/or data acquisition method. Bland Altman analysis indicates that 95% of pairs of measurements are predicted to have ratios between 3.73 and 0.05 indicating a very large random variability between the T2*/R2* method and the reference standard.

Results: A plot of the T2*/R2* LIC against the SDPA T2-MRI LIC (Figure 1) shows the vast majority of the data falling below the line of equivalence indicating that the T2*/R2* method is underestimating the LIC relative to the SDPA T2-MRI validated reference standard. The geometric mean ratio of T2*/R2* LIC to SDPA T2-MRI LIC was 0.44 (95% CI 0.36 –0.55) indicating severe underestimation of LIC by the T2*/R2* method. The geometric mean ratios of the two LIC measurements were significantly different for the two scanners (0.28 for Philips and 0.68 for Siemens, p <0.0001) indicating that the bias of the T2*/R2* method against the reference standard is not universal but is dependent on both/either scanner type and/or data acquisition method. Bland Altman analysis indicates that 95% of pairs of measurements are predicted to have ratios between 3.73 and 0.05 indicating a very large random variability between the T2*/R2* method and the reference standard.

Table 1.

<table>
<thead>
<tr>
<th>LIC Threshold</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;7 mg Fe/g dw</td>
<td>0.10 (95% CI -1.00)</td>
<td>0.23 (0.09 - 0.44)</td>
</tr>
<tr>
<td>&gt;15 mg Fe/g dw</td>
<td>0.09 (0.89 - 1.00)</td>
<td>0.40 (0.26 - 0.54)</td>
</tr>
</tbody>
</table>
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 NPIVs 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: 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 NPIVs 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.

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SIMILAR TRENDS IN RENAL FUNCTION AS MEASURED BY SERUM CREATININE DURING LONG-TERM IRON CHELATION TREATMENT WITH OR WITHOUT DEFERASIROX IN PATIENTS WITH TRANSFUSIONAL HEMOSIDEROSIS
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Background: Regular transfusion and iron chelation therapy (ICT) are often indicated for patients with β thalassemia, sickle cell disease (SCD) and other anemias, and can be lifelong requirements. As most patients now survive into adulthood and many experience prolonged exposure to ICT, there is increased risk of age-, disease- or drug-related complications, including changes in renal function. Evidence suggests that some patients receiving ICT experience changes in markers of renal function, mostly within normal limits, non-progressive and reversible with dose reduction and/or interruption. Recently, we report-ed a retrospective analysis of patients with transfusion-dependent anemias during a decade of deferasirox treatment indicating stable and a lack of any progressive worsening of renal function (Origa R et al. Blood 2016).

Aims: To assess serum creatinine (SCr) during long-term deferasirox treatment in subgroups of Italian patients with transfusional hemosiderosis who participated in the deferasirox registration studies and were then followed retrospectively.

Methods: Italian patients with β thalassemia, SCD, myelodysplastic syndromes or other anemias who received ≥1 deferasirox dose in the registration studies (studies 105, 106, 107, 108 or 109), had ≥1 post-baseline (BL) SCr measurement, and had medical records available were included. SCr values were collected retrospectively in 3-month periods from registration trial end until the latest patient assessment. Primary endpoint was SCr over time. SCr values during the retrospective period were evaluated by subgroups: here we report those who received only deferasirox and those who received no deferasirox but other ICT during the retrospective period.

Results: 282 patients were included in the retrospective study who received ≥1 deferasirox dose in registration studies; of these, during the retrospective period, 98 (35%) received only deferasirox (group A) and 82 (22%) received no deferasirox but other ICT (group B). In group A, mean (SD) age at first quarter was 25.9 (12.1) years and 36 (37%) were male; in group B, mean (SD) age at first quarter was 27.0 (10.9) years and 25 (40%) were male. The proportion of pediatric patients was 28% (n=27) in group A and 19% (n=12) in group B.

Mean (SD) duration of deferasirox exposure in group A was 7.5 (1.7) years; mean daily deferasirox dose was 4.3 (1.2) mg/kg. In both subgroups analyzed, mean SCr was within normal limits and remained stable over time during the retrospective period (Figure 1). Analysis in adults showed mean SCr values were stable over time. As expected in growing children who are gaining height and weight, pediatric mean SCr absolute values increased from baseline in proportion with an almost linear increase in muscle mass over time.

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.

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WHEN IRON LEADS TO RED CELLS (AND VICE VERSA): A COMPREHENSIVE PHENOTYPE -TOWARDS NGS/WS PATHWAY FOR THE DIAGNOSIS OF RED CELL AND IRON DISORDERS
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Background: Despite thorough clinical and biological phenotypic investigations, a number of red cell disorders remain uncharacterized. In addition, some of them are accompanied by overt iron overload and can be initially misdiagnosed as disorders of genes involved in iron metabolism. However an initial complete clinical, biological and morphological assessment is mandatory to correctly orientate towards the correct gene panel and to interpret the various molecular variations identified. We have set up in our center, a specialized outpatient consultation for both iron and red cell disorders. Specialized phenotypic investigations including biological and morphological tests followed by standard or second level genotyping can be prescribed.

Aims: The aims of this study was to characterize the molecular background of patients with iron or red cell disorders, or with a possible combination of both, and to propose key steps towards this genetic diagnosis.
Aims: 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 standard HFE genotyping 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 iron homeostasis or hereditary anemias, using NGS. Libraries were obtained using the Custom SureSelectXTarget Enrichment system (Agilent, Santa Clara CA USA) and sequenced on a MiSeq platform (Illumina, San Diego, CA, USA). Each deleterious variation was independently checked using conventional Sanger sequencing. Written informed consent was obtained from all the patients for NGS genetic analyses.

Results: Initial phenotypic reassessment allowed classifying the patients into 5 different groups: 1/ isolated hyperferritinemia (n=11); 2/ HF and IO (MRI >90 μmole/g dry weight) (n=17); 3/ hemolytic 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 chronic fully compensated hemolytic systemic 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 or C282Y/H63D genotype and another “iron gene” was also shown in 3 patients with IO (without anemia or hemolysis). No sequence variation of clinical significance was found in the sequenced genes of eleven of the studied patients.

Summary/Conclusions: On the phenotypic point of view, the present study highlights the importance to check for hematological data (CBC and reticulocytes) in patients with HF, because this can allow discovering fully compensated hemolysis and bringing towards a red cell disorder. On the other hand, it also underlines the importance to systematically check for IO all patients with a red cell disorder, who may display high LIC. Our present genotypic data (and previous data) suggest the frequency of combined inherited disorders of iron and red cells, making the combined search for both disorders quite relevant in clinical practice. This is now possible with the use of NGS analysis, which allows sequencing large numbers of genes. For those patients with no identified mutation, approaches using whole exome or genome can be proposed as the next step.

Method: Changes in liver iron concentration R2 MRI measurement across different chelation regimens in patients with haematological disorders: Real-life experience from LINCnet.

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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.

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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 analyzed by the University of Minnesota Medical Center, Fairview CLIA-certified clinical laboratory. Liver iron concentration measurements were made using single spin density projection-assisted R2-MRI (FerrScan®). Linear regression was used to determine the relationship between SF and LIC. Receiver operating characteristic (ROC) curve analysis was used to assess the sensitivity and specificity of SF concentrations for predicting LIC.

Results: The average age of the cohort (6 females and 11 males) at their first SF/LIC measurement was 18.3 years (range 9 to 30.3 years). Acute lymphoblastic leukemia (N=5) and acute myeloid leukemia (N=4) were the most common diagnoses and 15 of the 17 survivors had 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 an LIC above 15 mg Fe/L and a SF cut-off of 1076 mg/L has poor diagnostic performance for predicting a LIC above 7 mg Fe/L.

Table 1. ROC Curve Analysis.

<table>
<thead>
<tr>
<th>LIC Threshold (mg Fe/g)</th>
<th>SF (mg/L)</th>
<th>Sensitivity (PPV %)</th>
<th>Specificity (NPV %)</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 15</td>
<td>1270</td>
<td>1.00 (0.81 – 1.00)</td>
<td>0.50 (0.37 – 0.63)</td>
<td>0.92 (0.86 – 0.96)</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>1076</td>
<td>0.68 (0.48 – 0.81)</td>
<td>0.80 (0.53 – 0.90)</td>
<td>0.90 (0.82 – 0.96)</td>
</tr>
</tbody>
</table>

Note: AUC, area under the receiver operating characteristic curve.

Conclusion: Linear regression was used to determine the LIC 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. A SF cut-off of 1270 mg/L potentially has good sensitivity and specificity for predicting an LIC above 15 mg Fe/L, while a SF cut-off of 1076 mg/L has poor diagnostic performance for predicting an LIC above 7 mg Fe/L.

Figure 1.
**Methods:** Iron Sucrose complex (ISC) regarding improvement in haematological parameters and side effects in women with iron deficiency anaemia (IDA).

**Aims:** To compare safety and efficacy of Ferric Carboxymaltose (FCM) with Iron Sucrose complex in treatment of iron deficiency anaemia with minimal risk of release of large amounts of ionic iron in the serum.

**Results:** On day 0, 7, 14 & 28, Hb increment ≥3 g/dl seen in 63.33% and MCV>80FL seen in 100% of FCM group vs 0% and 43.33% in ISC group. FCM group had 3.17 g/dl increment in Hb vs 1.9 g/dl in ISC group. Ferritin increased to 147 ng/ml in 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.

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**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, P=5.12×10-10) and for nodular sclerosis HL (NSHL) at 3q28 (rs4459895, LPP=9.49×10-13), 5q33 (rs9298977, AH1, P=4.62×10-11), 10p14 (rs3781093, GATA3, P=9.49×10-13), 13q34 (rs112988813, UFP3A, P=4.58×10-8) and 16p13 (rs34972832, CLEC16A, P=1.29×10-8). Additionally, independent loci within the HLA region were observed for NSHL (rs2698018, 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 (AHI, P_MMR=8.63×10-6) and 10p14 (GATA3, P_MMR=4.70×10-6) and 10p14 (SMR=8.63×10-6) and 10p14 (SMR=4.70×10-6). Across new and established risk loci we confirmed a significant enrichment of DNase hypersensitivity in GM12878 cells (P=1.20×10-5), as well as regulatory elements in primary B-cells (P=6.0×10-5) and MCL (P=6.85×10-5). Analysis of ChiP-seq data on 82 transcription factors (TFs) in GM12878 cells, showed an over-representation of the binding of TFs that play a central role in B-cell signalling networks such as RELA (nuclear factor NF-kappa B p65), EBF1 (early B-cell factor 1), RUNX3 (run-related transcription factor 3) and BATF (basic 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.

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**SOX11 PROMOTES TUMOR PROTECTIVE MICROENVIRONMENT INTERACTIONS IN MANTEL CELL LYMPHOMA**

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1Human and experimental functional oncomorphology, IDIBAPS, 2Human and experimental functional oncomorphology, IDIBAPS, 3Vasculitis Research Unit, Department of Autoimmune Diseases, IDIBAPS, Hospital Clinic, University of Barcelona, 4Human and experimental functional oncomorphology, Department of Immunology, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain

**Background:** Mantle Cell lymphoma (MCL) is one of the most aggressive...
phoid neoplasms characterized by highly infiltrated tumor cells in lymphoid tissues and extra nodal sites. The patients have short responses to current therapies and frequent relapses. However, recent studies have identified a subset of MCL with indolent clinical behavior that tends to present with leukemic disease instead of extensive nodal infiltration, and that is characterized by the absence of the transcription factor SOX11 (SRY [Sex determining region-Y]-box 11). SOX11 oncogenic pathways 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 generated a stable transduced SOX11-silenced MCL cell line with reduced SOX11 protein levels by infecting MCL cell lines with lentiviral particles carrying shRNA plasmids specifically targeting SOX11. SOX11-positive MCL cell line was infected with the empty vector and used as a control. These two MCL cell lines were injected in two different mice models to analyze in vivo the role of SOX11, subcutaneously (sc) and peritoneally (ip) xenograft tumor models. To analyze the crosstalk between MCL and microenvironment, we did in vitro co-cultures 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 and conferred 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 technology, we assessed the enrichment of SOX11 in tumor repressor genes (CpGs) and promoter regions involved in these signatures, between them PDGF. 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 vasculature. Inhibition of PDGFA on endothelial cells was sufficient to inhibit tumor xenograft tumor models. SOX11-positive MCL and the primary cases of SCID mice with a PDGFA inhibitor reduced tumor growth and angiogenesis of SOX11-positive MCL xenograft tumors. We also observed that SOX11 promotes migration, pseudoemperiploisis (migration of tumor cells beneath stromal cells) and cell adhesion mediated-drug resistance (CAM-DR) in MCL cells, increasing cell proliferation and survival, and that these mechanisms were reduced in SOX11-negative cells. In the iv mouse model, we observed that SOX11-positive cells were able to migrated and infiltrated bone marrow and lymph nodes, whereas SOX11-negative cells were retained in peripheral blood.

**Summary/Conclusions:** In conclusion, our results show that SOX11 is regulating essential processes involved in aggressiveness of MCL tumor cells, as angiogenesis, invasion and drug resistance. Inhibition of SOX11-target genes may represent an efficient strategy for the treatment of aggressive MCL.
was measured and animals were randomly distributed into drug or vehicle group. At this time point mice were treated with 5mg/kg of selinexor or vehicle via oral gavage three times a week; subsequently, bioluminescence was assessed twice a week. Treatment with selinexor significantly increased mice survival, with a median survival of 48 days in the treatment group compared to 34 days in the vehicle group (*p<0.0001; Figure 1A). Mice in the treatment group could be distinguished from mice with slower increase in luminescence with two-way ANOVA (*p<0.0001; Figure 1B). Specific time-point analysis showed that differences were significant as soon as 8 days after treatment. At final point, histopathological analysis showed diffuse infiltration in meninges and cerebral parenchyma of highly proliferative CD20-positive B-cells. Currently, we are evaluating the synergy between ibrutinib and selinexor in vivo. For that we have used the same experimental setting and assigned 12 mice to each of the following groups: selinexor only (5mg/kg three times a week via oral gavage), ibrutinib only (25mg/kg daily in drinking water), combination or vehicle. Results will be available at the time of the meeting.

**Figure 1.**

**Summary/Conclusions:** Selinexor inhibits proliferation and survival of DLBCL cell lines regardless of COO and it can synergize with ibrutinib. Treatment of mice with CNS confined ABC-DLBCL with selinexor significantly reduces tumor growth and increases survival. Our results provide pre-clinical evidence for the development of selinexor as new therapeutic option for PCNSL or DLBCL with CNS involvement.

**P299**

**MOLECULAR HETEROGENEITY IN PERIPHERAL T-CELL LYMPHOMA NOT OTHERWISE SPECIFIED REVEALED BY COMPREHENSIVE MUTATIONAL PROFILING**

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**Background:** Peripheral T-cell lymphomas (PTCLs) are a highly heterogeneous group of mature T-cell neoplasms. In particular, accounting for the majority of PTCL, PTCL-not other specified (PTCL-NOS) is a diagnosis of exclusion and its biological role and natural history is poorly understood. In fact, recent genetic studies have suggested that a subset of PTCL-NOS is closely related to angioimmunoblastic T-cell lymphoma (AITL); both lymphoma types show follicular helper T-cell (TFH) phenotypes and share mutational targets in common, such as RHOA, TET2, DNMT3A, and IDH2. However, with the lack of comprehensive genetic analyses, the molecular pathogenesis is poorly understood in the majority of PTCL-NOS cases.

**Aims:** The aim of this study is to clarify a landscape of somatic mutations in PTCL-NOS.

**Methods:** We performed whole-genome exome and transcriptome sequencing of PTCL-NOS and other related PTCLs, followed by targeted-capture sequencing of candidate drivers in T cell lymphomas in 100 PTCL-NOS samples.

**Results:** Consistent with previous reports, TET2 (38%) was the most frequently mutated gene in PTCL-NOS, followed by RHOA (28%), TP53 (18%), KMT2C (13%), IDH2 (11%), and PLCG1 (11%). Frequent altered genes included signal transduction molecules (such as RHOA, PLCG1, STAT3 and SOCS1), chemokine receptors (CCR4 and CCR7), epigenetic modifiers (TET2, KMT2C, IDH2, DNMT3A, CREBBP, and KDM6A), and molecules associated with immune evasion (HLA-A, HLA-B, B2M, and CD58). Novel targets of recurrent mutation were also identified, including PDCD1, YTHDF2, and LRPP1B, which were frequently targeted by nonsense and frameshift mutations distributed throughout the entire genes. Among these, PDCD1 encodes PD-1, which transmits an inhibitory signal from PD-L1 and PD-L2 ligands, and therefore loss of function of this gene is predicted to enable malignant T-cells to escape from the negative signaling. By contrast, recurrent mutations in YTHDF2 and LRPP1B mutations in T-cell lymphoma-gene expression is unexpected. These genes encode a reader protein of N6-methyladenosine (YTHDF2), and a member of the low density lipoprotein receptor family (LRPP1B). Although the function of these genes in T-cells are unknown, our findings suggest their unresolved roles, whose dysfunction may lead to malignant T-cell proliferation. Finally, we investigated the co-occurrence between frequently mutated genes in PTCL-NOS. In accordance with previous observation, mutations characteristic of TFH lymphomas (TET2, RHOA, IDH2, and DNMT3A) tended to co-occur in a subset of PTCL-NOS cases, but were also almost exclusively with mutations in TP53 and chemokine receptor genes. These observations further support the molecular distinction between TFH and non-TFH lymphomas in PTCL-NOS: the former is more related to AITL and discriminated from the latter in terms of their mutational profiles.

**Summary/Conclusions:** In summary, our findings illustrate the landscape of somatic alterations in PTCL-NOS and provide a novel insight into their genetic and molecular heterogeneity, which should help to devise a novel molecular classification of PTCLs and to exploit a new therapeutic strategy to combat these intractable T-cell malignancies.

**P300**

**A COMPREHENSIVE PORTRAIT OF THE DNA METHYLMODE OF 866 SAMPLES FROM DIFFERENT B CELL NEOPLASMS: BIOLOGICAL INSIGHTS AND CLINICAL APPLICATIONS**

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**Background:** In the last years, a large body of evidence has been accumulated demonstrating that DNA methylation is not only widely altered in B-cell lymphoid tumors (and cancer in general) but it is also defining cell lineage and maturation stage. However, an integrative study of the whole DNA methylation of neoplastic B cells from different maturation stages has not been performed yet.

**Aims:** The aim of this study was to extensively dissect the dynamics of DNA methylation in B-cell neoplasias in the light of normal B cell maturation program. The ultimate goal of this study was to generate new clinically relevant knowledge with diagnostic and prognostic value.

**Methods:** Our dataset included whole-genome bisulfite sequencing data (n=57) and high-density methylation arrays (n=1161) from acute lymphoblastic leukemia (ALL), mantle cell lymphoma (MCL), Burkitt lymphoma (BL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) patients as well as from ten different normal B cell subpopulations. As DNA methylation estimates in neoplastic samples are influenced both by tumor cell content and composition of the micro environment, we developed a new method to deconvolute and in silico purify the methylation signal of tumors arising in different niches (bone marrow, peripheral blood an lymph node). The data were analyzed by a series of bioinformatic and biostatistical approaches and correlated with clinical variables.

**Results:** The initial bioinformatic approach to purify DNA methylation signals in B cell tumors revealed that samples with less than 55% tumor cell content could not be accurately purified. This strategy reduced the initial 1,044 tumor samples to 866. An unsupervised principal component analysis of in silico purified data revealed that each type of B-cell neoplasms clusters separately. ALLs clustered closer to precursor B cells, CLL and MCL closer to mature B cells and both DLBCL and MM showed the largest deviation from normal B cells. We then performed a differential analysis on the methylation data 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 outcome of the patients. Furthermore, for each tumor entity, we could identify from 5 to 19 epigenetic biomarkers that could classify each entity with high sensitivity and specificity.

**Summary/Conclusions:** In this study, we show that in silico purification of DNA methylation data is a powerful strategy to accurately measure DNA methylation alterations in tumor cells. Using a large dataset, we have developed a set of epigenetic biomarkers with high differential diagnostic power and identified that the epigenetic drift is a universal prognostic factor that can be applied to different B cell tumors.
ACTIVATION OF RHOA-VAV1 SIGNALING AXIS IN ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subset of peripheral T-cell lymphoma with follicular helper T-cell (TFH) features. We and others previously found mutations of RHOA, encoding p.Gly17Val (G17V RHOA mutation), with those in NF2 and IL-2RA, encoding IL-2 receptor alpha polypeptide. G17V RHOA mutation was found in up to 70% of AITL and other TFH lymphoma (a subgroup of peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS)) samples. RHOA, a small GTPase, is converted from the GDP-bound inactive form to the active GTP-bound form by guanine nucleotide exchange factors (GNEFs). The G17V RHOA mutant has been shown to be defective in RHOA-directed signaling, because it does not bind GTP. Therefore, it has remained unknown how G17V RHOA is involved in lymphomagenesis. VAV1 serves as an important mediator of T-cell receptor (TCR) signaling pathway through its GEF-dependent and -independent function. VAV1 activation is tightly regulated by tyrosine phosphorylations in the unstimulated state. Phosphorylation of VAV1 occurs within seconds in response to antigen stimulation of the TCRs by Syk and Src-family tyrosine kinases and initiates downstream TCR signaling.

Aims: We aim at clarifying the downstream signaling of the G17V RHOA mutation in AITL.

Methods: Proteomic screening was performed to identify G17V RHOA-specific binding partners. Binding was validated by co-immunoprecipitation of G17V RHOA and the candidate partners. Simultaneously, RNA sequencing was performed for 9 PTCL samples, including 6 AITL and 3 PTCL-NOS. Targeted deep sequencing of VAV1 was performed for 126 PTCL samples, including 69 AITL and 57 PTCL-NOS, 37 of which had RHOA mutations. The specific binding partner proteins of the G17V RHOA mutant were examined by high throughput screening in Jurkat cells. Nuclear factor of activated T cell (NFAT) activity in response to TCR stimulation was examined in Jurkat cells expressing wild-type (WT) and G17V RHOA mutant, and WT and various VAV1 mutants. The G17V RHOA mutant was examined by high throughput screening in Jurkat cells expressing wild-type (WT) and G17V RHOA mutant, and WT and various VAV1 mutants. Whole transcriptome was compared in Jurkat cells independently expressing each cDNA, in conditions with or without TCR stimulation. Expression of phospho-Vav1 was examined by immuno staining for AITL/TFH lymphoma samples.

Results: Proteomic screening identified the VAV1 protein as a G17V RHOA-binding partner. RNA sequencing identified a fusion gene involving VAV1 and STAP2 in an AITL sample without RHOA mutations. Moreover, targeted sequencing of VAV1 identified 2 in-frame deletion mutations in an ACID region (c.518–529del:p.173_177del and c.494–500del:p.165_174del) in AITL samples and 2 missense mutations in a zinc finger and SH3-SH2-SH3 module (c.G1668C:p.Glu556Asp and c.G1844T:p.Pro615Leu) in PTCL-NOS and AITL samples, respectively. Phosphorylation of VAV1 at Tyr 174 was enhanced in Jurkat cells expressing the G17V RHOA or VAV1-STAP2 cDNA than those expressing each WT cDNA or mock. Phosphorylation was blocked by the dasatinib at 1-10 nM concentrations. The G17V RHOA, VAV1-STAP2 and various VAV1 mutants enhanced NFAT reporter activities and interleukin-2 (IL-2) mRNA levels compared to their WT or mock. Vav1 was blocked by dasatinib and inhibitor such as GEMSTONE2, a small molecule inhibitor of ribosome. The G17V RHOA mutant was blocked by dasatinib or compound T at 1-10 nM concentrations. Finally, phospho-VAV1 was co-blocked by the dasatinib treatment. The levels of IL-2 mRNA were higher in VAV1 mutants enhanced NFAT reporter activities and interleukin-2 (IL-2) mRNA expression at the concentration range similar to dasatinib. The G17V RHOA, VAV1-STAP2 and various VAV1 mutants enhanced NFAT reporter activities and interleukin-2 (IL-2) mRNA levels compared to their WT or mock. Gene set enrichment analysis showed that cytokine and chemokine-related pathways were enriched in Jurkat cells expressing the G17V RHOA compared to those with WT or mock. Finally, phospho-VAV1 was co-blocked by the dasatinib treatment.

Summary/Conclusions: The G17V RHOA and VAV1 mutants both intensify the TCR pathway pathway through hyper-phosphorylation of Vav1. Our data suggest that the RHOA-VAV1 axis in AITL/TFH lymphoma may contribute to their clinical features and stand as a possible new therapeutic target.

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STAT3 IS CONSTITUTIVELY ACTIVATED AND CAN BE A THERAPEUTIC TARGET OF JAK INHIBITORS IN CHRONIC ACTIVE EPISTINE-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 aim.

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 SNTB, SNT15, SNT16 and the NK-cell lines SNK1, SNK6, SNK10 were examined. EBV-positive T or NK cell lines were obtained 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, 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-negative 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 cell lines from CAEBV patients revealed that the expression of STAT3-responsive genes, including interferon-γ were 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 cell lines 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.

P303

RECURRENT MUTATIONS IN MICRO-RNA BINDING SITES MAY BE POTENTIALLY RELEVANT IN FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) is the most common low grade B cell malignancy accounting for ~20% of all non-Hodgkin lymphomas. Approximately 30% of the FL cases suffer a histological transformation to a much more aggressive subtype of lymphoma drastically reducing the overall survival from 10 years to just 14 months. Despite being a critical event during disease progression it is molecularly poorly understood and no biomarkers exist to predict this phenomenon. Previous studies suggested the possibility that deregulation of microRNA expression (miRNAs, small endogenously produced non-coding RNAs) could be implicated in the development of FL disease as well as in the transformation event. We hypothesise that mutations in miRNA binding sites may also have a role in this process.

Aims: We evaluated the role of miRNA expression and potential miRNA targets in circulating lymphoma derived miRNA samples and cell lines from FL patients.

Methods: We interrogated whole genome sequencing (WGS HiSeq, Illumina) data from sequentially obtained samples of 6 FL patients that underwent trans-
formation using a bespoke bioinformatic pipeline based on TargetScan prediction algorithm in order to identify mutations in putative miRNA binding sites. Once identified, in order to validate them and test their recurrence in an extended cohort (60 samples from 31 FL patients who underwent transformation plus 21 samples of non-transformed FL patients) we designed an Ampliseq (Ion Torrent, Life Technologies) NGS custom panel. Finally, we selected a number of variants for assessing the variant effect on the miRNA:mRNA interaction by means of a combination of an in silico predictive algorithm and in vitro luciferase assays.

Results: 36% of somatic variants from WGS data arose in 3’UTR, and 68% of these were putative miRNA-binding sites (525 mutations in 497 genes). Interestingly, the ontology analysis showed that these mutations were not randomly distributed but rather there was enrichment in genes associated with haematological malignancies (P=2.18x10^{-4}). We then validated 85% of these mutations using targeted resequencing and found a total of 103 recurrent variants located in putative miRNA binding sites. QC criteria filtering led us to prioritise 38 variants in 25 genes to be functionally tested. Crucially, ontology analysis showed that these genes were highly enriched for GC-like B-cell lymphoma genes (P=4.39x10^{-5}), strongly suggesting that these variants may have a biological significance in the disease. We then performed an in silico approach based on TargetScan miRNA target prediction algorithm to evaluate the effect of the mutations on the binding of the miRNAs to their target sites. Based on these results we prioritized some of these genes to perform luciferase assays. We experimentally demonstrated not only that the majority of these loci are bona fide miRNA targets sites, but also that the presence of a number of these variants causes a dysregulation of the normal miRNA regulatory activity (Figure 1).

Results for luciferase assays. Figures A and B show an abrogation of the miRNA binding due to the effect of the mutations.

***p < 0.0001

Figure 1.

Summary/Conclusions: Our data show that the identified mutations do not occur randomly, but preferentially in putative microRNA binding sites of genes related to lymphomagenesis, supporting their role in FL pathogenesis. Furthermore, the presence of some of the identified variants in miRNA binding sites indeed promotes a dysregulation of the normal miRNA regulatory activity, suggesting that they might have a biological significance in FL.

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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 235x), and NGS variants were validated in 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) were associated with an increase in the hazard of progression both in univariate analysis as well as after adjusting for MIPI, Ki67 and blastoid variant: HR 3.87 (95% CI 1.64 to 9.13), p<0.002 and HR 3.66 (95% CI 1.77 to 7.56), p<0.001, respectively. These results translated into an increase of the hazard of death in both TP53 and KMT2D mutated patients both in univariate analysis as well as adjusting for MIPI, Ki67 and blastoid variant: HR 4.26 (95% CI 1.34 to 13.57), p=0.014 and HR 3.09 (95% CI 1.07 to 8.86), p=0.036, respectively. On these bases, a survival model was proposed based on the TP53 and KMT2D mutation status: 3-years PFS and OS were 26% and 64% for patients carrying either TP53 or KMT2D mutations or both vs 75% and 92% for patients without any of these mutations (Figure 1).

Summary/Conclusions: The updated clinical results of the FIL-MCL0208 trial show that: i) both TP53 and KMT2D mutations independently associate with shorter PFS and OS in younger MCL patients receiving high-dose therapy; ii) KMT2D mutations seem to be as detrimental as TP53 mutations, at least in terms of PFS; iii) given the negative prognostic impact of these mutations, they might be used to select high-risk patients for novel therapeutic approaches.
Multifaced aspects of bleeding disorders

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A LOOKBACK AT VWD TYPE 2A AND 2M CLASSIFICATION IN A LARGE COMPREHENSIVE HAEMOPHILIA CENTRE.

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Background: von Willebrand Disorder (VWD) has a prevalence of approximately 1% in the general population and is due to quantitative deficiencies or qualitative defects of the Von Willebrand Factor (VWF) protein. VWF is a large multimeric protein with multiple functions. It carries and protects factor VIII and helps in the binding of FXIII, 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 quantitative deficiencies (Type 1 and 3) and VWD type 2 are qualitative defects of the VWF protein with or without quantitative deficiency as well. Type 2 VWD is further subdivided into type 2A, 2B, 2M and 2N. These subtypes depend on a number of laboratory assays that measure the FVIII activity, VWF protein level (VWFAg assay) and the function of the protein i.e its ability to bind to 1) FVIII (VWF binding assays), 2) platelets (VWF Ristocetin assay) and 3) collagen (VWFCB assay). Other tests include ristocetin induced platelet aggregation (RIPA), multimer analysis, assay ratios and VWF genetic analysis. No single commercially available laboratory method can achieve to test all the parameters required to clinch the accurate diagnosis of the subtypes of VWD. Use of these triple assays with VWF cofactor/FVIII ratio, VWF CB (VWF-CB) and 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 ristocetin, Platelet agglutination method, VWF CB Elisa methods, VWF multimeric analysis by gel chromatography and VWF exon 27/28 genetic mutations are routinely done. New information and new set of results for the registered patients have been taken into account the classification of VWD type 2A and 2M and the database is updated.

Results: In the VWD database 36 patients classified as 2M and 19 patients as type2A have been recorded from 2000 to end of 2016. With the updated results and genetic analysis and the response to DDAVP, around 30% of the patients have had their subtypes changed. This exercise confirms that no singular test can be used to accurately diagnose the VWD and its subtypes and illustrates the importance of DDAVP testing and the difficulty of interpreting assay ratios for VWF fragments. These levels are <1% normal.

Summary/Conclusions: VWD may be misdiagnosed, underdiagnosed or undiagnosed. Appropriately and complete investigative panel is necessary for complete classification of VWD and its subtypes.

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RETROSPECTIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF DYSFIBRINOGENEMIA AND HYPODYSFIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS

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Background: Dysfibrinogenemia (DF) and hypodysfibrinogenemia (HDF) patients pts) experience hemorrahgs 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 was 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 complications. Haemorrhagic events 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.
patient with the rarest occurrence of 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), paroxonase (PON1 Gln192Arg), methyleneetetrahydrofolic reductase (MTHFR C677T), catalase (CAT C-262T) and plasmatic glutathione peroxidase (GPX3 T-165C) was studied by PCR-RFLP technique. Statistical differences between the patient and control group were assessed by Fisher’s exact test. Odds ratios (OR) with their 95% confidence intervals (CI) and p-value were calculated by using GraphPad Prism 5.0 software.

Results: We found abnormal distribution of ApoE genotypes in the patient group. Absence of ApoE e3 allele was observed in 7 (9.9%) men with SH and 9 (3.1%) controls (OR=3.4, 95% CI: 1.2-9.7, p=0.025). In particular, the frequency of ApoE e2/e2 genotype was 10-fold increased in patients when compared to healthy men (4.2% vs 0.4%, OR=11.2, 95% 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 0.2%, respectively). In the patient group, we observed the positive association between the PON1 192Gln/Gln variant and heterozygous GPX3 -65TC genotype (OR=5.8, 95% CI: 1.3-25.7, p=0.021). Simultaneous presence of these genetic variants was more than 5-fold frequently found in SH than in controls (8.5% vs 1.6%, OR=5.5, 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 MOMP-2 AND MOMP-9 IN PATHOGENESIS OF INTRACEREBRAL HEMORRHAGE IN CONGENITAL FACTOR XII DEFICIENCY
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Background: Congenital factor XIII deficiency (CFXIIID) is a rare bleeding disorder. Intracerebral hemorrhage (ICH) is a leading cause of mortality and morbidity in this disorder. Matrix metalloproteinase-2 (MMP-2) and MMP-9 are involved in pathogenesis of intracerebral hemorrhage (ICH) and their methylation status of this gene did not correlate with the clinical course of ICH with particular correlation between the first hemorrhage and severity of ICH. We intended to apply diagnostic exome sequencing (DES) for genetic confirmation and finding causative variants in children with primary hemostatic defects.

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 MPRA probeix 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 (GP1BA, GP1BB, GP9 VWF, MYO5A, RAB27A, MLPH, GPX3), inhibitors (PLAU, VPS33B, RAB27A, MLPH), syndrome of dysglobulinemia (LYST, HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DNTBP1, BLOC1S3, Chediak-Higashi syndrome (LYST), Criscelli 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.1451G>A (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.399GT (p.Pro113 Gly118delArg) 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: 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.
Myelodysplastic syndromes – Clinical 1

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AN ALGORITHM TO IDENTIFY CASES OF SEVERE HEMORRHAGE IN ROUTINELY COLLECTED HEALTHCARE DATA
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Background: Many patients with a hematological malignancy have an increased risk of hemorrhages. Research addressing the causes of these hemorrhages, especially those on major hemorrhages, are hampered by the difficulty to find sufficient and representative cases of major hemorrhage. Unfortunately, electronic health records generally do not codify hemorrhages.

Aims: The aim of this study was to develop an algorithm that can be used to find patients who suffered from major hemorrhages (WHO grade 3 or 4) within electronic health records.

Methods: An algorithm was developed using electronic health record data of a cohort of patients with acute leukemia, who received platelet transfusions between June 2011 and December 2015 at the Leiden University Medical Center in the Netherlands. Chart review was performed for a stratified, random sample of observation days. Discriminative performance of three indicators was assessed: CT-brain, drop in hemoglobin level and transfusion need within 24 hours. The cut off values for hemoglobin drop and transfusion need with the best discriminating capacity and CT-brain were entered in the final algorithm. The C-statistic was calculated and calibration plots were made. The algorithm will be externally validated in two other academic hospitals.

Results: The derivation cohort consisted of 255 patients comprising 10,638 observation days and chart review was performed for 353 days. The incidence of major hemorrhage was 0.22 per 100 observation days. The final algorithm consisted of information on CT-brain (yes/no), a hemoglobin drop of ≥2.8 g/dl and the need of six or more transfusions (yes/no). The C-statistic of the algorithm was 0.93 (95% confidence interval (CI) 0.86 to 0.99). The incidence of bleedings with all grades of severity was 8.4 per 100 days. The algorithm for bleedings of all grades had a c-statistic of 0.54 (CI 0.53 to 0.55). The results of the external validation are not available yet.

Summary/Conclusions: An algorithm using information on CT-brain, hemoglobin drop and transfusion can accurately identify cases of major hemorrhage within electronic health care data. External validation will be performed.

P313
MOLECULAR MECHANISMS AND CLINICAL SIGNIFICANCE OF REDUCED PTPN1 EXPRESSION IN MYELODYSPLASTIC SYNDROMES
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Background: Previously we determined common deleted region (CDR) of del(20q) observed in MDS by CGH-array. Our data showed that the PTPN1 gene is located within CDR of del(20q). The PTPN1 gene encodes PTP-1B, a non-receptor type protein tyrosine phosphatase, which is involved in multiple physiological and pathological cellular processes via dephosphorylation of several tyrosine kinases, and other molecules. Although roles of PTP-1B in normal and pathological hematopoiesis has not been elucidated, it may function negative regulator for cellular processes mediated by tyrosine kinases, including JAK2, and SRC. We hypothesized that the PTPN1 gene is a target gene disrupted by del(20q), resulting in haplo-insufficiency, and involved in MDS molecular pathogenesis.

Aims: We attempted to examine PTPN1 expression level in bone marrow cells of MDS patients with or without del(20q), and to investigate its clinical and biological significance.

Methods: Total RNA was extracted for cDNA synthesis from bone marrow samples taken at the time of diagnosis with written informed consent from patients and control subjects were used for the present study. Real-time RT-PCR was carried out to quantify PTPN1 expression by the TaqMan probe method using an ABI 7500 real-time PCR system (Applied Biosystems). Data including patients’ demographic, disease status, medical history, clinical and laboratory findings, and outcome, were collected from medical records and laboratory data base. A non-parametric Mann-Whitney-Wilcoxon test was used to examine whether expression levels among groups are statistically different. The Kaplan-Meier model was used to analyze the impact of PTPN1 expression on overall survival, and log-rank test was used for statistical analysis. We also examined the effect of 5-azacytidine treatment on PTPN1 expression in primary bone marrow cells of MDS patients. Bone marrow cells were cultured with or without 5mM of 5-azacytidine for 48 hours. Expression level of PTPN1 was examined by quantitative RT-PCR described as above.

Results: A total of 118 MDS patients, 71 males and 47 females with median age of 68 years (range: 20-91 years) and 19 control subjects were included in the present study. The patients were classified as RCUD (n=18), RCDM (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, RCDM, RARS, RAEB-1, and RAEB-2 were 1.52, 1.95, 1.91, 1.46, and 1.26 respectively. Relative PTPN1 expression level in WHO-subtypes with high blast counts (RAEB-1 and RAEB-2) was significantly lower than that in WHO-subtypes with less blast counts (RCUD, RCDM, RARS) (median value: 1.41 vs 1.89, P=0.0074). To investigate prognostic implication of PTPN1 expression in MDS, we analyzed impact of PTPN1 expression on overall survival (OS). Based on PTPN1 expression level, 118 patients were divided into four groups, high (Q1), intermediate (Q2), Q3, and low (Q4) quartiles. Kaplan-Meier analysis demonstrated that the lowest quartile (Q4) showed significantly worse survival compared with remaining quartiles (Q1, Q2, Q3) (P=0.048). The estimated 5-year OS rates in Q1-3 group and Q4 group were 69% and 49.8%, respectively. We examined whether PTPN1 expression is induced by 5-azacytidine in primary bone marrow cells of 17 MDS patients. Real-time PCR analyses indicated that 5-azacytidine treatment significantly induced PTPN1 expression.

Summary/Conclusions: The present study demonstrated that PTPN1 expression is reduced in MDS patients by 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.
Background: DNA hypomethylating agents (HMAs) comprise standard therapy for non-transplant candidate high-risk myelodysplastic syndromes (MDS). However, little is known about the actual 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 MDS patients in which the efficacy was compared between the 5- and 7-day regimens. A total of 107 patients were enrolled between 2013 and 2016. For all cases, a bone marrow specimens collected before treatment was analyzed for mutations using targeted-capture sequencing. Mutations were then interrogated after 4 cycles of azacitidine therapy in 48 (45%) cases. An additional 12 cases were analyzed for mutations who received azacitidine therapy for MDS and whose bone marrow specimens were available both before and after therapy. RNA baits were designed for detection of both 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-2006 criteria. The size of clones showing the maximum allelic burden between pre- and post-treatment specimens (ΔTFC: tumor cell fraction).

Results: On average, 2.7 mutations (range 0-9) were detected per sample before azacitidine treatment. TP53 represented the most common mutation with a prevalence of 40% (20/50) and 15% (2/13) being mutated in the on- and off-protocol cohort, respectively, followed by ASXL1, RUNX1, TET2, and SRSF2. TP53-mutated cases had significantly lower number of driver mutations (1.7 vs 3.1/sample, p<0.001) and higher number of copy number changes (9.6 vs 2.1, p=0.001), compared with unmutated cases. Clinical response was observed in 25 cases in the on-protocol cohort, including 6 complete remission (CR) (3.6%) and 19 major CR (mCR) (17.8%) and 7 (29%) cases (all CR) in the off-protocol cohort. Notably, CR was obtained almost exclusively in TP53-mutated cases (5/6 and 5/7 CR cases in the on- and off-protocol cohort. No other mutations were associated with complete remission. Median treatment duration was 119 days (range: 81–721), which lasted for a median duration of 217 days (range 10 –783). ΔTFC was evaluable for 62 cases who had one or more follow-up specimens and carried at least one mutation in either pre- or post-treatment with an average of –0.075 (range: –0.75–0.72). ΔTFC was significantly lower in responders than non-responders (–0.18 vs –0.0002, p=0.0068) and in TP53-mutated cases (–0.25 vs 0.0058, p=0.006).

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 used for high-risk MDS patients. In conclusion, TP53-mutated MDS patients showed a better response to azacitidine therapy compared 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 TP53 mutations were in CR at the final evaluation. Seven (21%) out of 33 evaluable patients achieved a complete cytogenetic response. Ten (20%) subjects with TP53 mutations were in CR at the final evaluation.

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

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/m² 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 were AML and MDS to decitabine. Clinical response was observed in 25 cases (15 of them 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 TP53 mutations were in CR at the final evaluation. Seven (21%) out of 33 evaluable patients achieved a complete cytogenetic response. Ten (20%) subjects with TP53 mutations were in CR at the final evaluation.
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).

Summary/Conclusions: This study confirms the benefit of AZA treatment on outcome in patients with HR-MDS and cytogenetic abnormalities involving chromosome 7.

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UN UPDATE OF A PHASE II EXPLORATORY STUDY OF OPN-305, A TOLL-LIKE RECEPTOR 2 ANTIBODY, IN PATIENTS WITH LOWER RISK MEYLODYPLASTIC SYNDROMES WITH PRIOR HYPOMETHYLATING AGENT THERAPY
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Background: Alterations of innate immune signaling, including overexpression of TLR2, are common in MDS. Significant TLR2 overexpression in MDS bone marrow CD34+ cells, especially after HMA therapy, has been reported. OPN-305 is a fully humanized antagonistic IgG4 kappa monoclonal antibody to TLR2 which significantly increases the formation of erythroid colonies (CFU-E) in BM CD34+ cells isolated from pts with lower-risk MDS in vitro.

Aims: Evaluate the potential therapeutic value of OPN-305 in patients (pts) with MDS.

Methods: We designed a phase II/III trial of OPN-305 for pts with Low or Int-1 risk MDS by IPSS after failure to prior therapy with a HMA (34 cycles).Pts whose dose 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 10mg/kg was planned for N=30 pts.

This initial cohort allowed evaluation of toxicity, pharmacokinetic analysis, receptor occupancy, and sequential analysis of cytokine profile. An extension dose escalation phase to 10mg/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 normocellular, 2 (16%) had high, 8 (67%) had bone marrow aspirates was complete in virtually all samples taken after OPN-305 administration. There is no evidence of alloreactive (ALUR) or auto-antibodies. Compared with baseline, no significant changes of IL-23, IL-18, IFN-r, IL-10, IL-18, IL-6, IL-12 (p40), IL-12 (p70) and IL-8 levels where observed among responders or non-responders or based on OPN-305 dosing. A trend to increased response was observed in patients with higher TLR2 expression, though this was less evident based on cytogenic risk.

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% transduction improvement, and potential association between TLR2 levels and response.

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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 significance.

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 ACGAs 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 RUCUD, 2 (1.8%) RARS, 22 (19.50%) RCMRD, 28 (24.8%) RAEB-1, 32 (28.3%) RAEB-2, 12 (10.6%) Unclassifiable MDS, 9 (8%) CMMML 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 CMMML (10.6%) were categorized in the HR level. Among patients with known karyotype (101 of 115 patients) 1 (11.3%) had a complex karyotype (CK). Regarding mutational analysis, 44 patients (38.3%) didn’t show any mutation before transplant; 27 patients (23.5%) had 1 mutated gene, 15 (13%) had 2, 19 (16.5%) had 3 (6.2%) had 4, 3 (3.2%) had 5 and only 1 patient (0.9%) had 6 different mutated genes. The most frequently mutated genes were: TP53 in 15 patients (13%), SRSF2 in 14 (12.2%), SET2 in 13 (11.3%), DNMT3A in 9 (7.8%), RUNX1 in 9 (7.8%), SF3B1 in 9 (7.8%) and ASXL1 in 8 (7%) patients. After a median of follow up for survivors of 2.02 years, Overall Survival (OS) was 48.1% (63.4% at 1 year; median 5.96 years). Patients were divided into 2 groups: group 1 with 2 or less mutated genes and group 2 with more than 2 mutated genes. Compared with group 1, group 2 patients had a higher OS (46.9% vs 69.6% at 1 year; p=0.035) and a higher Cumulative Incidence of Relapse (CIR) (25.3% vs 10.1% at 1 year; p=0.007). Development of cGVHD significantly improved outcome in both groups (Figure 1). Univariate analysis determined that developing of cGVHD, CK, number of mutated genes and mutations in SET2 significantly impacted on outcome. Nevertheless, only the development of cGVHD as a time-dependent variable (HR 0.046, 95%CI 0.016-0.138, p<0.001) and TE2 mutations (HR 2.562, 95%CI 1.018-6.447, p=0.046) significantly influenced on OS in multi-

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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.

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VOSAROXIN PLUS AZACITIDINE TREATMENT FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME: A PHASE 1/COHORT EXPANSION STUDY

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Background: Although hypomethylating agents are the mainstay of treatment for myelodysplastic syndromes (MDS), these agents result in remissions in a minority of patients and are not curative. Vosaroxin is a first-in-class quinolone derivative that intercalates DNA and inhibits topoisomerase II. Vosaroxin is active with a tolerable safety profile in acute myeloid leukemia (AML) and the novel combination of vosaroxin and azacitidine was found to be synergistic in primary myeloblasts.

Aims: This phase 1/cohort expansion study was designed to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of vosaroxin when given in combination with azacitidine, and to evaluate the efficacy and safety of the combination treatment.

Methods: Patients with MDS ≥18 years old with cytopenias requiring transfusions, an IPSS score of intermediate (INT)-1 or greater, or chronic myelomonocytic leukemia were eligible. Vosaroxin (initial dose: 50 mg/m²/d) was administered on Days 1 and 4, and azacitidine (75 mg/m²/d) on Days 1-7 of a 28-day cycle, in an outpatient setting, for up to 6 cycles in a 3+3 design (additional cycles were permitted if a clear benefit for the patient was demonstrated). Once the MTD was determined, an expansion cohort of 20 evaluable patients (≥1 cycle) was enrolled.

Results: A total of 35 patients enrolled in the dose escalation (n=13) and expansion (n=22) phases. The median age of the entire cohort was 66 years (range 38-77) with IPSS scores of low (n=1); INT-1 (n=13); INT-2 (n=15); and high risk (n=6). The median ECOG score for the entire cohort was 1 (range 0-2). In the dose escalation phase, at the initial dose of vosaroxin 50 mg/m²/d (n=6), the median number of total cycles was 2 (range: 1-4); 2 of 6 patients experienced a DLT at this dose (grade 4 hyperbilirubinemia and grade 4 neutropenia >42 days). At the de-escalated dose of 34 mg/m²/d (n=7), the median number of cycles was 2 (range: 1-18); 1 patient experienced a DLT at this dose (grade 4 mucositis). The MTD of vosaroxin was determined to be 34 mg/m²/d when given on Days 1 and 4 with a fixed dose of 75 mg/m² of azacitidine on Days 1-7. The major non-hematologic toxicities were infections, febrile neutropenia, and bleeding. The combination of vosaroxin and azacitidine showed promising activity with responses rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.

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 response rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.
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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 (IMiD: 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 Pomalidomide (Pom), as well as two XBP1 truncating mutations in PI refractory patients. Of note, among three treated cases with mutations in the β5 (PSMB5) or βi (PSMB8) PI binding subunit of the proteasome, one patient harbored not less than 4 subclonal mutations. This is the first description of PSMB5 mutations in human MM, identified in a patient with long term history of PI treatment. All mutations were located in or close to the Bor binding site of PSMB5. The functional analysis demonstrated induction of resistance not only to Bor (IC50wt= 2 nm vs IC50mut= 4.5-8 nm), but also to the second generation PI Ixazomib (IC50wt= 5.2 nm vs IC50mut= N/A) and Carfilzomib (IC50wt= 8 nm vs IC50mut= 13-22 nm). Of interest, the P97 blockade of the protein homeostasis by the investigational compound CB5083 remains still possible in the mutated cell lines and the resistance can be overcome. Finally, Pom treatment eradicated two of the PSMB5 containing subclones (Figure 1).

Summary/Conclusions: Under the selective pressure of anti-MM therapy the incidence of mutations in genes associated with drug resistance increases in patients resistant to multiple drugs. Resistance mechanisms evolve in parallel in competing (sub)clones of the disease, mimicking phenotype and behavior. Remarkably, despite our restrictive gene selection, a quarter of our treated cohort is affected by at least one mutation. Aim of future therapy may be the eradication of selected clones or subclones, which, according to our data, appears possible.

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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 regions 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. We functionally validated, both in vitro and in vivo, Interleukin-2- enhancer binding factor 2 (ILF2) as a key 1q21 amplification-specific gene. Our results show that ILF2 interacts 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 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

Figure 1. A: Mutation incidence in IMID related and PI related genes. B: Functional analysis of PSMB5mut expressing AMO-1 cells with different PI inhibitors and the P97 inhibitor CB5083.
effectors, which enables genomic instability, promotes adaptive mechanisms to genotoxic stress, and enhances cell survival, thereby promoting drug resistance and disease progression. Given that 1q21 amplification is one of the most frequent copy number alterations in cancer, synthetic lethality approaches based on targeting gain-of-function associated with ILF2 may have a broad spectrum of application to potentiate the sensitivity of cancer cells to chemotherapeutic agents.

Figure 1.

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PROGNOSTIC IMPLICATION OF SOMATIC MUTATIONS BY NEXT GENERATION SEQUENCING: AN ANALYSIS FROM THE MMRF COMMPASS STUDY IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: High throughput techniques, such as next generation sequencing, are becoming an appealing approach to characterize multiple myeloma (MM) genomic profiles and better define risk assessment. However, the clinical relevance of such approaches is still largely unknown. The Multiple Myeloma Research Foundation (MMRF) CoMMpass trial (NCT01454297) has collected data from 1154 newly-diagnosed MM patients enrolled worldwide. Comprehensive analysis of somatic mutations in MM cells at diagnosis could unravel prognostically relevant disease characteristics not detectable with traditional approaches.

Aims: We analyzed data from the interim analysis 8 cohort (August 2015) to create a prognostic model.

Methods: CD138+ purified MM specimens from bone marrow aspirates and peripheral blood cells were collected at diagnosis. Whole exome libraries from both tumor and constitutional DNA samples were created. Somatic single nucleotide variants (SNV) were identified, only nonsynonymous SNV were included in the analysis. We evaluated the impact on progression free survival (PFS) of recurrently mutated genes (with at least a nonsynonymous SNV with 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 (high risk, standard risk and missing). A backward selection based on the Akaike Information Criterion (AIC) was used to identify the final Cox model used to create a scoring system.

Results: 517 patients with baseline somatic mutation data were included in the analysis. Median age at diagnosis was 64 years (range 27-93), all patients received novel agents as first line treatment, 236 (45.6%) received autologous stem cell transplantation (ASCT). The most recurrent mutated genes were KRAS (25%) and NRAS (19.5%). Consistently with other works, DNA allele frequency data revealed that, in the great majority of cases, only a subclonal portion of MM cell DNA harbors a selected somatic SNV (data not shown). Based on the impact on PFS of recurrently mutated genes, a scoring system was developed. Four groups were identified according to the mutational status of 9 genes selected in 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.

Figure 2.

Summary/Conclusions: The use of a prognostic model based on the mutational status of 9 recurrently mutated genes could improve risk assessment of newly-diagnosed MM patients. Longer follow-up and validation in independent cohorts of patients are needed to confirm our findings. Updated results with a longer follow-up will be presented at the meeting.

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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 CR91 technology (TRI-ACT). We used 14 cell lines including MM, lung, and breast cancer cell lines. We designed a pooled library targeted shRNA/CR91 screen containing 8 shRNAs along with their matched control for each of the 500 genes in the 1q21.23-3 region, including IncRNA and miRNA in addition to protein coding genes. The pooled library contained 6500 shRNAs, including CR91 control. We measured proliferation and nucleosome positioning (chromatin expression profiling) for the expression of CRBN and subsequently the development of IMiD resistance, as well as investigate whether restoration of sensitivity to IMiDs is feasible through epigenetic reprogramming by epigenetic modulators. Lenalidomide and pomalidomide was primarily associated with a global expression changes in either promoter DNA methylation or chromatin accessibility. We then treated the IMiD-resistant cell lines with the combination of 5-Azaazacytidine (a DNMT1 inhibitor) and EPZ-6438 (an EZH2 inhibitor) for 48 hours, before exposing the cells again to IMiDs, and found that resistant cell lines treated with this combination of 5-Aza and EPZ-6438 showed significantly increased apoptotic response, similar to the sensitive cell lines. Even more interestingly, we found that the treatment with 5-Aza and EPZ-6438 almost completely restored the global chromatin accessibility changes associated with acquired IMiD resistance back to the initial state, even though the mechanism remains to be further investigated. 5-Aza and EPZ-6438 was also effective in sensitizing the majority of cell lines with intrinsic resistance to IMiDs. We also observed that treatment with the combination of 5-Aza and EPZ-6438 failed to induce a significant upregulation of CRBN in the IMiD-resistant cell lines, thus suggesting that the process of degradation of 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-Azaazacytidine and an EZH2 inhibitor, which makes 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.
Background: The signaling lymphocytic activation molecule family 3 (SLAMF3) is a member of the immunoglobulin superfamily expressed on T, B, and natural killer cells and modulates the activation and cytotoxicity of these cells via self-ligand binding. SLAMF3 is also expressed on plasma cells from patients with multiple myeloma (MM), although its role in MM pathogenesis remains unclear.

Aims: To clarify this, we investigated the expression and functions of SLAMF3 in MM.

Methods: 1) Two hundred thirty patients comprising 153 newly diagnosed (19 asymptomatic and 134 symptomatic) MM patients, 30 refractory/relapsed MM patients, and 47 patients with monoclonal gammopathy of undetermined significance were enrolled. SLAMF3 and CD138 expression levels on clonal plasma cells were analyzed using flow cytometry (FCM). Soluble SLAMF3 (sSLAMF3) serum levels were measured using ELISA. 2) Drug sensitivity toward antimalleoma agents (melphalan and bortezomib) and the proliferation potential in MM cell lines KMS18 and U266 were analyzed using FCM and the MTT assay. SLAMF3 knockdown MM cell lines were obtained using the lentiviral shRNA system and siRNA. Stable transfected KMS34 cell lines overexpressing full-length SLAMF3 and cytoplasmic domain-truncated SLAMF3 (ΔSLAMF3) were established through corresponding vectors. Single-nucleotide polymorphism (SNP) genotyping was analyzed by real-time PCR. The adaptor protein of SLAMF3 was identified by Western blotting and immunoprecipitation.

Results: 1) SLAMF3 was highly expressed on plasma cells in almost all MM patients, even in relapsed/refractory disease, although CD138 expression levels were decreased in some with advanced disease. 2) The proliferative potential and percentage of antimalleoma agent-induced apoptosis in SLAMF3ΔC-terminal cells were significantly higher and lower than in SLAMF3ΔC-terminal cells, respectively. The cell proliferation and drug resistance in SLAMF3-expressing KMS34 cells were promoted in comparison with SLAMF3ΔC-terminal cells. That malignant potential in MM cells was cancelled by SLAMF3 knockdown. Furthermore, the proliferation of MM cells and resistance to antimalleoma agents were inhibited by anti-SLAMF3 antibody. Adaptor proteins, SHP2 and GRB2, were expressed in MM cell lines, and their expression levels were decreased in some with advanced disease. 3) The proliferative potential of MM cells and resistance to antimalleoma agents were inhibited by SNPs associated with IL2, TNFA, CD154, and CD200 signaling pathways.

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advanced stage significantly more often and had shorter progression-free survival times than those with low levels (p=0.012, \( \chi^2 \)).

Summary/Conclusions: This study revealed that SLAMF3 molecules consistently expressed on MM cells may transmit positive signals mediated via the complex of SHP2 and GRB2 by self-ligand interaction between MM cells and induce a high malignant potential in MM. Furthermore, high levels of serum sSLAMF3 may reflect MM disease progression and be a useful prognostic factor in MM. Thus, SLAMF3 molecules may be a new potential target for future immunotherapy and chemotherapy.

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TARGETING CD74 IN MULTIPLE MYELOMA WITH A NOVEL ANTI-BODY 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-to-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 EC50 and percent span of killing in MM cell lines and xenografts. An exploratory toxicology study was conducted in a non-human primate model.

Results: In vitro cytotoxicity assays show nanomolar potency of STRO-001 in MM cell lines and reduces tumor burden in MM xenograft models, including significant prolongation of survival in the MM.1S model. Based on these encouraging observations, STRO-001 is advancing to the clinic for the treatment of CD74-expressing B-cell malignancies.

Summary/Conclusions: STRO-001 demonstrates potent in vitro cytotoxicity in MM cell lines and reduces tumor burden in MM xenograft models, including 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.

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GENOTYPE CHARACTERIZATION OF LIGHT CHAIN AMYLOIDOSIS BY WHOLE EXOME SEQUENCING


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Background: Immunoglobulin light-chain amyloidosis (AL) is a heterogeneous and multifactorial disease with high genetic complexity. Until now, no common factor or unique mutation associated with this disease has been described. Whole exome sequencing in Multiple Myeloma (MM) patient’s tests allowed to know important genes and pathways that are involved in the disease. However, few evidences through next generation sequencing (NGS) analysis were described in AL. Consequently, the application of NGS technologies permits unraveling the genomic landscape of AL to better disentangle the biology of the disease, allowing the identification of new therapeutic targets as in MM.

Aims: Genotype characterization of novel molecular alterations in AL plasma cell by whole-exome sequencing technology.

Methods: We studied 40 paired samples (sorted pathological plasma cells and peripheral blood) from 20 patients with AL. Whole exome and regulatory regions were captured using Agilent’s SureSelect Human All Exon V6+UTR kit and sequenced on the Illumina NextSeq 500 platform with pair-end sequencing technique with a global mean depth coverage of 70x, on target coverage of 96.5% and a Phred quality score of 91.3% up to Q30. Data were analyzed with Strelka software 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 76 (range 18-177) mutations per patient. 28.4% of the mutations was located on regulatory regions (5' UTR, 3' UTR). So far, we did not identify recurrent mutations between the patients, although some patients presented different mutations on the same gene. 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), 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 gammapathies - Clinical 1

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IMPROVED SURVIVAL IN 21,465 MULTIPLE MYELOMA PATIENTS: RESULTS FROM A POPULATION-BASED STUDY
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Background: Multiple myeloma (MM) is generally considered an incurable disease, however advances in the treatment options for MM have been great in recent years. Recent studies on these new agents indicate an improvement in survival, nevertheless population-based studies have had contradicting findings, especially in the elderly patients.

Aims: The aim of the study was to evaluate the survival of all patients diagnosed with MM in Sweden in the years 1973 to 2013 and to relate the survival pattern to trends in treatment strategies.

Methods: Patients diagnosed with MM in the period from January 1, 1973 to December 31, 2013 were identified from the Swedish Cancer Registry. Information on sex, date of birth, date of diagnosis, and date of death was collected. Relative survival ratios (RSRs) were used to provide a measure of excess mortality of MM patients compared to a comparable group from the general population. RSRs with 95% confidence intervals (CIs) were found for 1-, 5-, and 10-years for 4 calendar periods: 1973-1982, 1983-1992, 1993-2002, and 2003-2013 and furthermore for 6 age categories at diagnosis (0-40, 41-50, 51-60, 61-70, 71-80 and >80). Short-term survival, as defined by RSR of less than 3 months, was also defined for all calendar periods.

Results: A total of 21,465 patients (54% males, median age at diagnosis 72 years) with MM were recorded in the time period. Overall, the 1- and 5- and 10-year RSRs improved in the whole period, with the greatest improvement in the two most recent calendar periods. The 1-year RSR increased significantly between all calendar periods (0.69, 0.74, 0.77 and 0.82, respectively). The 5-year RSR increased significantly between the two last calendar periods (0.31, 0.33 and 0.41, respectively; Figure 1) as well as the 10-year RSR (0.10, 0.12, 0.14 and 0.20, respectively). Short-term survival increased significantly between the first two and last two calendar periods (the RSR were 0.83, 0.88, 0.89 and 0.93 respectively). Females had a lower excess mortality compared to males (excess mortality ratio 0.91).

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

Figure 1.

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 tetrassomies 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 375 HRA in 327 patients (28% of the cohort): 170 (45%) del(17p), 110 (29%) t(4;14), 45 (12%) t(14;16), 8 (2%) t(14;20), and 42 (12%) gain(1q). Of the 280 patients with 1 HRA 130 (46%) had del(17p), 120 (43%) had a high-risk translocation, and 30 (11%) had gain(1q). Of the 46 patients with 2 HRA 34 (76%) had del(17p) and a high-risk translocation, 6 (13%) had a high-risk translocation and gain(1q), 5 (11%) had del(17p) and gain(1q), and 1 had 2 high-risk translocations. There was 1 patient with 3 HRA: del(17p) and t(4;14) and gain(1q). The median overall survival was 6.6 years (6.0 - 8.0) for the entire cohort (n=1181), 8.3 years (6.7 - 8.9) for those without HRA (n=854, 72%), 4.8 years (3.9 - 5.6) for those with one HRA (n=280, 24%), and 2.7 years (2.1 - 3.8) for those with 2 or more (2+) HRA (n=47, 4%). Figure 1 shows the Kaplan-Meier overall survival estimates stratified by the number of HRA (n=1181). The presence of 1 HRA (versus 0, HR 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).

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.
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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 vs PBO 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=153) showed the treatment effect is not dissimilar to the ITT analysis (HR 0.53; 95% CI, 0.25-0.76). Sensitivity analyses showed consistent results. Updated data will be presented at the meeting.

Table 1.

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.

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UPDATED RESULTS FROM ASPIRE AND ENDOVAIR, RANDOMISED, OPEN-LABEL, MULTICENTRE PHASE 3 STUDIES OF CARFILZOMIB IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: D. Siegel1, 2, A. Orlo1, P. Rajnic3, J. Minarik4, V. Hungria5, J.H. Lee6, K. Song7, 8.5.2% and 2.0% for cardiac failure, and 2.4% and 8.6% for peripheral neuropathy for KRd and Rd, respectively. In ENDEAVOR, 929 patients were randomised. Baseline characteristics were balanced between arms. At a median follow-up of 19.4 months (Kd) and 17.7 months (Vd), median PFS was 17.6 months (Kd) and 9.4 months (Vd) (HR: 0.53; 95% CI: 0.44–0.63; P <0.0001); 18-month PFS rates were 48.7% (Kd) and 23.9% (Vd). Median OS in Kd was 57.4 months (100% censor rate) and 10.2 months (Vd) (HR: 0.50; 95% CI: 0.42–0.60; P <0.0001). Median TTNT was 26.1 months (Kd) and 14.5 months (Vd) (HR: 0.49; CI: 0.40–0.60; P <0.0001). 15.8% (Kd) and 14.9% (Vd) of patients discontinued because of AEs. Grade ≥3 AE rates were 13.8% and 3.3% for hypertension, 5.2% and 2.0% for cardiac failure, and 2.4% and 8.6% for peripheral neuropathy for KRd and Rd, respectively. In ENDEAVOR, 929 patients were randomised. Baseline characteristics were balanced between arms. At a median follow-up of 19.4 months (Kd) and 17.7 months (Vd), median PFS was 17.6 months (Kd) and 9.4 months (Vd) (HR: 0.53; 95% CI: 0.44–0.63; P <0.0001); 18-month PFS rates were 48.7% (Kd) and 23.9% (Vd). Median OS in Kd was 57.4 months (100% censor rate) and 10.2 months (Vd) (HR: 0.50; 95% CI: 0.42–0.60; P <0.0001). Median TTNT was 26.1 months (Kd) and 14.5 months (Vd) (HR: 0.49; CI: 0.40–0.60; P <0.0001). 15.8% (Kd) and 14.9% (Vd) of patients discontinued because of AEs. Grade ≥3 AE rates were 13.8% and 3.3% for hypertension, 5.2% and 2.0% for cardiac failure, and 2.4% and 8.6% for peripheral neuropathy for KRd and Vd, respectively.

Summary/Conclusions: Consistent with the primary analyses, these results show that incorporation of carfilzomib into treatment regimens in patients with RRM is associated with clinically meaningful improvements in PFS and a favourable benefit-risk profile.

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EFFECTIVENESS AND SAFETY OF DARATUMUMAB, LENALIDOMIDE, AND DEXAMETHASONE VERSUS RD ALONE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA: UPDATED ANALYSIS OF POLLUX
M.A. Dimopoulos1, 2, P. Moreau2, H. Nahi3, T. Ploner4, H. Goldschmidt5, M. Obreja8, S. Aggarwal8, R. Hajek9, 40.53; 95% CI: 0.42–0.60; P <0.0001). Median TTNT was 26.1 months (Kd) and 14.5 months (Vd) (HR: 0.49; CI: 0.40–0.60; P <0.0001). 15.8% (Kd) and 14.9% (Vd) of patients discontinued because of AEs. Grade ≥3 AE rates were 13.8% and 3.3% for hypertension, 5.2% and 2.0% for cardiac failure, and 2.4% and 8.6% for peripheral neuropathy for KRd and Vd, respectively.

Aims: To provide updated efficacy and safety data from POLLUX, a multicenter, phase 3, randomized study of DRd versus Rd in RRMM.

Methods: Eligible patients with ≥1 prior line of therapy were randomly assigned to Rd (25 mg PO lenalidomide on Days 1–21 of each 4-week [Q4W] cycle) with or without daratumumab (16 mg/kg IV once weekly for Cycles 1 and 2, every 2 weeks for Cycles 3–6, then Q4W until disease progression). Patients who were refractory to lenalidomide were excluded. Progression-free survival (PFS) was the primary endpoint. Bone
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; POLLUX) significantly prolongs progression-free survival (PFS) in elderly patients (pts) with relapsed/refractory multiple myeloma (RRMM) and maintains 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 DVD group then continued to receive D monotherapy qw4 for 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 POLLUX (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) were similar with DVD and Vd (15% vs 20%). Common (≥10%) grade 3/4 TEAEs for Dvd were thrombocytopenia (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), 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 63%) were also higher with DRd than with Rd. 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.

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.

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Summary/Conclusions: The all oral ixThalDex regimen showed an ORR of 63% with no difference in pts with or 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

Background: Growth differentiation factor-15 (GDF-15), is a member of the TGFBeta 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 been 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 been shown to be correlated with early death and shorter survival independently of other cardiovascular and Mayo stage. Because GDF-15 was also associated with renal outcomes we evaluated the prognostic value of GDF-15 levels in two independent cohorts of patients with AL amyloidosis and renal involvement who were treated in two different centers (Pavia Amyloidosis Center and Department of Clinical Therapeutics, Athens).

Aims: To evaluate the prognostic value of GDF-15 levels in independent cohorts of patients with AL amyloidosis and renal involvement.

Methods: Circulating levels of GDF-15 were measured by a novel pre-commercial immunoassay (R&D Diagnostics) in stored serum. The Pavia cohort included 135 and the Athens cohort included 76 patients with AL amyloidosis and renal involvement. Standard criteria were used for the diagnosis, evaluation of organ involvement and amyloidoma-based risk stratification. Renal staging was based on the system proposed by Palladini et al., based on baseline proteinuria >5 gr/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=0.09), while 93% and 94% of patients in the two cohorts had GDF-15 levels >1200 pg/ml (the upper limit of normal for individuals without cardiovascular disease). We then evaluated the prognostic significance regarding renal outcomes (dialysis): GDF-15 >4000 pg/ml was associated with a HR of 6 (95% CI 2015.6, p=0.001) in Athens cohort (progression to dialysis within 2 years in 7% vs 47%); while, by applying the same cutoff in patients in Pavia cohort, 2-year dialysis rate was 10% vs 37% (HR: 3.95 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.
AN OPEN-LABEL, PHASE 2 STUDY TO EVALUATE THE ORAL COMBINATION OF IXAZOMIB, 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 ICd regimen in transplant-ineligible pts with NDMM. Primary endpoint was rate of CR+VGPR+PR during induction 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 22, for up to 13 28-day cycles as induction. Pts with ≥SD and an acceptable toxicity profile then received single-agent ixazomib maintenance therapy until PD, death, or unacceptable toxicity.

Results: 70 NDMM pts were enrolled (n=36 Arm A; n=34 Arm B): median age 73 years (range 61–87); 47% male; 31%/33%/29% ISS stage I/II/III MM; 50% had a cardiovascular/pulmonary comorbidity; 9% had high-risk cytogenetics (t(4;14), t(14;16), del 17p). At data cut-off (29 June 2016), pts had received a median of 19 cycles; 66% had completed 13 ICd induction cycles and proceeded to ixazomib maintenance therapy; 10% were ongoing on therapy, and 53% had discontinued due to AEs (24%), PD (18%), patient withdrawal (3%), or other reasons (10%). Confirmed responses by investigator assessment are shown in the Table 1. Median time to first/best response across arms was 2/4 months. After a median follow-up of 17.9/18.5 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 [31%]), anemia (19 [27%]), diarrhea, nausea, peripheral edema (each 18 [26%]), vomiting (15 [21%]), fatigue, and constipation (each 14 [20%]). The most common grade ≥3 AEs were neutropenia (22 [31%]), anemia (10 [14%]), lower respiratory tract and lung infections (9 [13%]), and supraventricular arrhythmias (5 [7%]). There were 5 on-study deaths, none considered related to treatment. QoL (by EORTC QLQ-State, 7St John Cancer Center, Medical University of Lublin, Lublin, Poland, subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, United States, 7St. John Cancer Center, Medical University of Lublin, Lublin, Poland, 8Department of Medicine, West Virginia University, Morgantown, United States, 9Department of Hematology, Hospital Clínic de Barcelona, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 10Department of Haematology, University Hospital Ostrava, Faculty of Medicine, Ostrava University, Ostrava, Czech Republic, 11Winship Cancer Institute of Emory University, Atlanta, United States

Table 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.

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THE ORAL PROTEASOME INHIBITOR IXAZOMIB IN COMBINATION WITH MELPHALAN-PREDNISONE FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: PHASE 1/2 DOSE-ESCALATION STUDY (NCT01335685)

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Background: Bortezomib-MP is a standard-of-care regimen for elderly NDMM pts. Whereas bortezomib is administered IV or SC, ixazomib is an oral proteasome inhibitor with a safety profile amenable to extended dosing that is approved in the US and EU, in combination with lenalidomide-dexamethasone, for the treatment of MM pts who have received at least 1 prior therapy. Based on the demonstrated feasibility and efficacy of a proteasome inhibitor-MP combination, the all-oral ixazomib-MP (IMP) regimen was evaluated in elderly, transplant-ineligible NDMM pts.

Aims: Primary phase 1 objectives were to determine the safety, MTD, and recommended phase 2 dose (RP2D) of ixazomib in combination with MP. The primary phase 2 objective was to determine the rate of CR+VGPR, secondary objectives included PFS and OS.

Methods: In phase 1, pts were enrolled to 4 arms – Arm A: ixazomib 3.0–3.7 mg (days 1, 4, 8, 11, 22, 25, 29, 32) plus M 9 mg/m2 and P 60 mg/m2 (days 1–4) in 42-day cycles (max 9 cycles); Arm B: ixazomib 3.0–4.0 mg (days 1, 8, 15) plus M 6 mg/m2 and P 60 mg/m2 (days 1–4) in 28-day cycles (max 13 cycles); Arm C: ixazomib 3.0–4.0 mg (days 1, 8, 15, 22, 29) plus M 4 mg/m2 and P 60 mg/m2 (days 1–4) in 28-day cycles (max 9 cycles). In phase 2, an expansion cohort was enrolled at the RP2D. On all arms, after IMP induction, pts could receive maintenance with single-agent ixazomib (days 1, 8, 15; 28-day cycles).

Table 1.

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-oral triplet including a PI and alykatori, 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 400 mg/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 (AEs, 21%). CR+VGPR rate was 43% (43% at RP2D), including 28% (22%) ≥CR and 19% (17%) sCR; median time to first response was 1.7 mos, and responses continued to mature over a long period (Table 1). Depth of response improved during ixazomib maintenance in 9/36 (25%) pts (VGPR to sCR in 5 pts; VGPR to CR in 2 pts; CR to sCR in 2 pts). Median TTP/PFS are shown in Table 1; median OS was not reached after median follow-up of 42.6/46.9 mos overall/at RP2D.

**Summary/Conclusions:**

The RP2D was weekly ixazomib 4.0 mg plus M 6 mg/m² and P 60 mg/m² (days 1–4) in 28-day cycles, consistent with the ixazomib dose and schedule in TOURMALINE-MM1. AEs were mainly hematologic, infections, PN, and diarrhea. The all-oral IMP regimen is active in NDMM, with a 28% CR rate (19% sCR), a 43% ≥VGPR rate, and a median PFS of 23.5 mos; responses continued to improve over a prolonged period.

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Myeloma and other monoclonal gammopathies - Clinical 2

### P340

**FEASIBILITY AND EFFICACY OF DOSE ADJUSTED MELPHALAN – BORTEZOMIB IN PATIENTS ≥75 YEARS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; PRELIMINARY RESULTS OF THE PHASE II HOVON 123 STUDY**


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**Background:** There is a high rate of toxicity-related discontinuation in elderly patients with NDMM, negatively affecting outcome. In order to predict feasibility of treatment the IMWG developed the frailty score based on age, (instrumental) Activities of Daily Living and the Charlson comorbidity index.

**Methods:** Patients were treated with 9 cycles of MPV: Mel 6 mg/m², day 1-4; Pred 30 mg/m², day 1-4; and Bort 1.3 mg/m² day 1,8,15 and 22 of a 35-day cycle. This first planned analysis was restricted to the first 140 consecutive patients out of 240 planned patients.

**Results:** Of the 139/140 eligible patients none were fit (because of age ≥75 years), 30/139 (22%) were unfit, 100/139 (72%) were frail, and 9/139 (6%) unknown. The median follow up was 17.0 months. The discontinuation rate of MPV in the total population was 42%; 27% in unfit and 46% in frail patients (p=0.09). When also patients were included who discontinued bortezomib only these numbers were 27% in unfit and 52% in frail (p=0.02). Importantly, 6 cycles of MPV were found to be feasible in 70% of patients, both in unfit (80%) and frail (69%) patients. Age >80 years was associated with a significantly higher discontinuation rate of MPV or bortezomib only (70% versus 35% in patients aged 75-80 years, p=0.01). WHO performance was not associated with discontinuation rate. Response on protocol was sPR 3%, ≥VGPR 38% and ≥CR 11%, not significantly different in unfit versus frail patients. Response after 6 cycles was sPR 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).

**Table 1.**

<table>
<thead>
<tr>
<th>Unfit patients</th>
<th>Frail patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>77 (75-80)</td>
</tr>
<tr>
<td>WHO (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>≥4</td>
<td>0</td>
</tr>
<tr>
<td>IS (%)</td>
<td></td>
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<tr>
<td>1</td>
<td>0</td>
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<tr>
<td>2</td>
<td>1</td>
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<tr>
<td>3</td>
<td>13</td>
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<td>4</td>
<td>27</td>
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<td>5</td>
<td>30</td>
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<td>6</td>
<td>35</td>
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<tr>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>≥8</td>
<td>0</td>
</tr>
<tr>
<td>Grip strength (N)</td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>9 (7 missing)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>34</td>
</tr>
<tr>
<td>Strong</td>
<td>37</td>
</tr>
<tr>
<td>Walking speed (m/s)</td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>9 (7 missing)</td>
</tr>
</tbody>
</table>

However, 58% and 59% of frail patients had an intermediate or high walking speed and grip strength respectively. Vice versa, 8% of patients with low
walking speed and 12% of patients with low grip strength, were not frail but unfit according to the IMWG frailty index. Disconnection 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 EFFICIENT AND SAFE IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA**

**Aims:** To assess the safety, tolerability and efficacy of the carfilzomib, bendamustine and dexamethasone (CBd) regimen in treatment-naïve patients with advanced multiple myeloma (MM) with ≥2 prior lines of therapy.

**Patients and Methods:** Sixty-three patients with RRMM with ≥2 lines of prior therapy were enrolled with the last patient included in February 2017. Treatment consisted of 28-day cycles of Benda 70 mg/m² on day 1 and 8, Carf was given on day 1, 2, 9, 15, 16 at 27 mg/m² after an initial dose of 20 mg/m². In 6 patients in the phase 1 part of the trial Carf was escalated to 36 mg/m². This was found to be the MTD. 20 mg dex was added on every treatment day and day 22 and 23. After 8 cycles, responding patients received maintenance only every 14 days with relapsed/refractory multiple myeloma (RRMM).

**Methods:** Thirty-six patients with RRMM with ≥2 lines of prior therapy were enrolled with the last patient included in February 2017. Treatment consisted of 28-day cycles of Benda 70 mg/m² on day 1 and 8, Carf was given on day 1, 2, 9, 15, 16 at 27 mg/m² after an initial dose of 20 mg/m². In 6 patients in the phase 1 part of the trial Carf was escalated to 36 mg/m². This was found to be the MTD. 20 mg dex was added on every treatment day and day 22 and 23. After 8 cycles, responding patients received maintenance only every 14 days with Carf for two days plus dex until progression.

**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 5.7 months. Number of prior treatment lines ranged from 2 to 9, and ≥85% of patients had received previous transplantation, 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 ≥grade 3 included 3 (2%) infections, 3 (2%) gastrointestinal events and thromboembolism. These data will be up-dated 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 signals were observed, they were generally manageable and did not influence treatment option. In this heavily immunosuppressed patient population infection prophylaxis is mandatory.

**Clinical trial information:** NCT02056756.

**P342**

**CHEMOTHERAPY BEFORE AND AFTER HEART TRANSPLANTATION FOR PATIENTS WITH REFRACTORY MULTIPLE MYELOMA: SINGLE CENTER RESULTS WITH LONG-TERM FOLLOW-UP**

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**Background:** Even after a prolonged treatment patients with multiple myeloma may have received little chemotherapy, however many may suffer from bortezomib induced peripheral neuropathy (PN). Bendamustine (Benda) leads to increased levels of defective ribosomal products (DRiPs). Carfilzomib (Carf), a proteasome inhibitor not inducing PN, inhibits degradation of DRiPs leading to plasma cell apoptosis.

**Aims:** With this scientific rationale a CBd combination of Carf and Benda and low dose maintenance dexamethasone was evaluated in a phase 1/2 trial in patients with relapsed/refractory multiple myeloma (RRMM).

**Methods:** Sixty-three patients with RRMM with ≥2 lines of prior therapy were enrolled with the last patient included in February 2017. Treatment consisted of 28-day cycles of Benda 70 mg/m² on day 1 and 8, Carf was given on day 1, 2, 9, 15, 16 at 27 mg/m² after an initial dose of 20 mg/m² in 6 patients in the phase 1 part of the trial Carf was escalated to 36 mg/m². This was found to be the MTD. 20 mg dex was added on every treatment day and day 22 and 23. After 8 cycles, responding patients received maintenance only every 14 days with Carf for two days plus dex until progression.

**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 5.7 months. Number of prior treatment lines ranged from 2 to 9, and ≥85% of patients had received previous transplantation, 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 ≥grade 3 included 3 (2%) infections, 3 (2%) gastrointestinal events and thromboembolism. These data will be up-dated 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 signals were observed, they were generally manageable and did not influence treatment option. In this heavily immunosuppressed patient population infection prophylaxis is mandatory.

**Clinical trial information:** NCT02056756.

**P343**

**MM-013 PHASE 2 MULTICENTER STUDY OF POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA AND RENAL IMPAIRMENT**

**Aims:** Further investigation of the tolerability and efficacy in pivotal RRMM trials in pts with moderate RI. However, this approach is not feasible in pts with severe RI requiring hemodialysis. All pts must have received ≥1 prior treatment 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 (177). With a median of 5 months after HTx (4-29), 18 patients received ASCT. HDM was used with either 200 mg/m² (N=10) or reduced dosage (N=8) in patients with reduced kidney function (mostly due to renal complications after HTx). Complete remission (CR) was achieved in 7 patients (24% of all transplanted pts, n=29, 2 patients have not finished treatment yet), very good partial remission (VGPR ≥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:** HTx can be performed by chemotherapy is a feasible treatment approach in patients with advanced cardiac amyloidosis. Patients who reach HTx have a nearly 50% chance for a very good hematologic remission (VGPR or better) and consecutively a favorable survival probability with a median OS of nearly 6 years in our series.

**Background:** RI is a common comorbidity in pts with multiple myeloma (MM) that increases in incidence as the disease progresses and is associated with poor prognosis. Approximately 20% to 30% of pts with MM have RI at time of diagnosis; 2% to 13% of whom will require dialysis. The immunomodulatory agent, POM, in combination with LoDex demonstrated comparable efficacy and tolerability in pivotal RRMM trials in pts with moderate RI. However, this regimen had not previously been fully studied in pts with severe RI or pts requir- ing hemodialysis.

**Aims:** To present updated safety and efficacy analyses from the multicenter, phase 2 MM-013 trial, in which pts with RRMM and moderate or severe RI, including those on hemodialysis, were treated with POM+LoDex.

**Methods:** Three cohorts of pts with RRMM and RI were enrolled: (A) – moderate RI (estimated glomerular filtration rate [eGFR] <30 to <45 mL/min/1.73m²), (B) – severe RI (eGFR <30 mL/min/1.73m²) without hemodialysis, and (C) – severe RI requiring hemodialysis. All pts must have received ≥1 prior treatment including LEN and progressed during or after their last anti-myeloma treatment before entering the study. Pts received POM+LoDex until disease progression
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 0.94 in both cohorts A and B, and 0.99 in cohort C. ORR was 39.4%, 29.4%, and 14.3% in cohorts A, B, and C respectively. PFS and OS results are presented in the Table 1. Grade 3/4 anemia and thrombocytopenia occurred more frequently in cohort C, likely due to severe RI requiring dialysis (Table 1). AEs leading to dose reductions were 18.2%, 14.7%, and 14.3% in cohorts A, B, and C respectively.

Table 1.

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

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 mobilising PBSC has yet to be defined, with either G-CSF alone, also called steady state procedure, or use of Plerixafor, a CXCR4 antagonist (Mozobil®) +G-CSF or high dose cyclophosphamide versus Plerixafor on behalf of IFM.

Aims: To determine the safety, tolerability, and antitumor activity of pembrolizumab monotherapy in patients with RRMM.

Methods: Patients with RRMM who have failed 2-3 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 2 (7%) patients were 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.

Table 1.

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 disease stabilization. Recent results of ongoing studies, such as KEYNOTE-023 (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 mobilising PBSC has yet to be defined, with either G-CSF alone, also called steady state procedure, or use of Plerixafor, a CXCR4 antagonist (Mozobil®) +G-CSF or high dose cyclophosphamide (usually administered at a dose of 1.5 to 6g/m² IV for 5 days), with or without GM-CSF, as the most commonly used mobilisation regimen, 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 costs to 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 underwent ASCT. This study was not aimed at evaluating the suitability or advisability of one therapy versus another. A cost-consequences analysis of the different regimens of mobilization was conducted. Costs were derived using a micro-costing approach, only direct medical costs are included in this economic analysis. 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. Following the best regimens, the credibility of their ranking was found by the simulation approach.

Results: Ten RCTs were identified including nine treatment regimens: vincristine-dexamethasone (VAD), bortezomib-dexamethasone (VD), bortezomib-cyclophosphamide-dexamethasone (VCD), bortezomib-thalidomide-dexamethasone (VTDP), bortezomib-thalidomide-dexamethasone-cyclophosphamide (VTDC), lenalidomide-dexamethasone (RD) and bortezomib-lenalidomide-dexamethasone (VRD). This analysis showed that (i) lenalidomide-dexamethasone was significantly better than VD, VCD, and VTD; (ii) probability of being best regimen (84% of the simulations).

Summary/Conclusions: For a long time HD cyclophosphamide was recommended for mobilization upfront in Myeloma therapy, as it was needed to improve response rate and depth of response, despite only 10% of the patients improving. With the progress made recently with the induction regimens, the choice for the mobilization regimen is now based more on safety and cost saving. In that regards, one must acknowledge that plerixafor has become one, if not the, most attractive option for Myeloma.

Figure 1. SYSTEMATIC LITERATURE REVIEW AND NETWORK META-ANALYSIS OF INDUCTION TREATMENT FOR NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA PATIENTS

P347
A STUDY OF UTILITY OR FUTILITY OF PERFORMING SKELETAL SURVEYS IN PARAPROTEINAEMIA: A MULTICENTER EXPERIENCE FROM UK
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Background: Recent International Myeloma Working Group (IMWG) guidelines recommend that conventional skeletal surveys should be supplemented 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 rise of pre-emptive imaging modality has led to a universal downsizing of skeletal survey.

Aims: To look at all skeletal surveys requested across 3 large hospitals in UK over a year and analyze their justification, effectiveness and utility.

Methods: A total of 397 skeletal surveys were performed across three hospitals over one year. The data set was analyzed for clinical indications, paraprotein level, rationale for requesting the skeletal survey, the diagnostic yield and also the number of follow up CT/PET or MRI required.

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 WBLCCT irrespective of the initial skeletal survey result, indicating a significant duplication rate and waste of resources. In the MGUS 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 MGUS group. However none these were positive. When the clinic-biochemical algorithm was applied, the number of requests was reduced by at least a quarter (24%), avoiding unnecessary radiation exposure and precious resources.

Summary/Conclusions: Our systematic review and NMA included most of the recommended induction treatments for transplant-eligible myeloma patients and identified VRD as being most effective in achievement of 3VGPR. NMA can provide an overview of the best treatment and each regimen’s relative efficacy in case of lacking head-to-head RCTs, thereby supporting clinical decision-making.

Figure 1. A STUDY OF UTILITY OR FUTILITY OF PERFORMING SKELETAL SURVEYS IN PARAPROTEINAEMIA: A MULTICENTER EXPERIENCE FROM UK

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 high-sensitivity methods (The Binding Site). Normal range for k/l FLCr was 0.26-1.25. We defined immunosuppression as levels of both the uninvolved (polyclonal) FLC+uninvolved heavy+light chain (HLC; measured with Helyelite) 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 205 African American patients, 37/196 (19%) had abnormal FLCr. 2/196 (1%) had elevated iFLC with immunosuppression identified in 23/196 (12%). Median PFS for MRD-negative patients was not reached; however both an abnormal FLCr (median PFS: 31.4 months; p<0.001) and immunosuppression (median PFS: 31.4 months; p=0.005) identified a group of patients with poorer outcomes. On the other hand, median PFS for MRD-positive patients was 21.3 months; 42(3%) 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 high-sensitivity MFC bone marrow assessment at the end of consolidation therapy render the most powerful prognostic information in MM patients achieving deep responses. In those where disease is no longer detected using MFC, abnormal FLCr confer poor prognosis, which may partly be due to inefficient immune recovery. Absolute FLC measurements were not informative, supporting the rationale of evaluating biomarkers of the tumour and immune system recovery. Our results warrant further studies to validate the clinical utility of FLC measurements in combination with next-generation (8-colours) flow cytometry.

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) (Avest-Loiseau, Leukemia, 2013). Historically, t(11;14) has been associated with standard-risk multiple myeloma (MM) and generally favorable outcomes (Avest-Loiseau, Leukemia, 2013). However, some retrospective 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 IDH1/2, diabetes history, and baseline levels of hemoglobin, platelets, calcium, and creatinine. Data cut-off 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).

Summary/Conclusions: In Connect MM, the effect of t(11;14) on OS was significantly different between African American and non-African American patients. Specifically, t(11;14) was associated with poorer survival outcomes in African American patients, and not in non-African American patients. Thus, the presence of t(11;14) may be a risk factor for poor prognosis in African American patients. Additional analyses will be conducted to elucidate the role of induction treatment, transplant and maintenance in African American and non-African American patients with t(11;14).

Table 1.
Myeloproliferative neoplasms - Clinical 1

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


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Background: Juvenile myelomonocytic leukemia (JMLL) is an aggressive myeloproliferative disorder of early childhood. While some cases show spontaneous remission, allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment option for the majority of patients, however, the 5-year event-free survival reaches only about 50%. Hyperactive RAS signaling is assumed to be the main driving event in JMLL. It is caused by genetic alterations in CBL, KRAS, NRAS, or PTEN in about 90% of patients. So far, there is no clear understanding of how RAS pathway mutations relate to the heterogeneous disease biology and variable clinical outcome seen in JMLL patients. As a consequence, established clinical and genetic markers fail to predict post-HSCT outcome for the vast majority of JMLL patients. Aims: We hypothesized that DNA methylation profiling, either alone or in combination with genetic alterations, might provide a molecular basis for disease classification.

Methods: Genome wide DNA methylation analysis using the HumanMethylation450 beadchip was performed in a discovery cohort of 20 JMLL patients. We developed a strategy to eliminate methylation events that attribute to epigenetic changes in normal hematopoiesis. The clinical relevance of our findings was assessed in an unselected sample set consisting of 148 consecutive patients with JMLL (n=130) or Noonan syndrome associated myeloproliferative disorder (n=18) registered in the EWOG-MDS 1998 & 2006 trials. Data integration was performed in a subset of patients with available exome sequencing (n=50) and expression profiling (n=15) data.

Results: Systematic DNA methylation analysis of JMLL samples identified three subgroups with low, intermediate and high methylation levels (LM, IM, and HM). Detailed analysis of the validation cohort not excluding the Noonan patients identified an association of methylation groupings with clinical features. The HM subgroup (n=41) was enriched for high-risk characteristics: All HM cases had elevated levels of HbF, 88% were older than 2 years at diagnosis, 74% had low platelets (<70×10⁹/l), and 66% carried somatic PTEN mutations. In contrast, the LM subgroup (n=62) was enriched for patients with low-risk disease: All 18 patients with Noonan syndrome, 13/14 patients with CBL syndrome, and 15/19 patients with NRAS mutations were assigned to the LM group. The IM group (n=45) was enriched for cases with monosomy 7 and somatic KRAS mutations. The unfavorable risk profile in the HM group translated into nearly 5-year survival (HM 57%, LM 77%, log rank p<0.01) and a high incidence of relapse after HSCT (HM 48%, LM 9%, Gray’s test p=0.01). In a multivariate Cox regression model, only methylation group (HM vs LM: RR 10.9 [1.8-66.2]) and PTEN/M11 mutation status (PTEN M11-mutant vs other: RR 3.3 [1.2-9.9]) remained as independent prognostic factors for CIR (p<0.01).

JMML patients with ≥2 somatic RAS-RAF-MEK-ERK pathway activating mutations were assigned to the LM or HM group. PTEN/mutation status (PTEN-mutant vs other: RR 3.3 [1.2-9.9], p<0.01) and expression profiling (n=15) data. 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 unknown. The most prevalent individual abnormalities were 20p- (20%), 13q- (20.8%), +8 (8.3%), +9 (5.6%), and +12 (5.6%). Patients with pre-PTP-MF had significantly higher frequency of abnormal karyotypes than those with post-PTP-MF (P<0.012). Chromosomal abnormalities did not cluster differently among the different genotypes (JAK2, CALR, MPL and triple negative). Abnormal karyotype was significantly associated with lower platelet count (P<0.004), larger spleen size (P=0.16), 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 in patients with MK was significantly lower than in patients with all other karyotypes (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, hemoglobin, platelets and bone marrow blasts at the time of sample collection for sequencing were 13.4 (1.1-79) 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 affected survival (p=0.063), and mutated ZRSR2 had a borderline 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 encoding molecules, thylus (JAK2, NRAS), the epigenetic modifiers (ASXL1 and SETBP1) were found at frequencies ≥10%. Although the analysis is limited by small numbers, EZH2 mutations were independently associated with poor survival. This represents the largest cohort of patients with MDS/MPN-U interrogated for mutations in multiple genes to date.

P353 MUTATIONAL LANDSCAPE OF MYELODYSPLASTIC SYNDROME/MYELOPROLIFERATIVE NEOPHAN - UNCLASSIFIABLE
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Background: MDS/MPN-U is a rare, poorly characterized myeloid neoplasm within the MDS/MPN category in the World Health Organization (WHO) classification. A median survival of 12.4 months from time of referral was previously reported for a cohort of 85 patients with MDS/MPN-U seen at the MD Anderson Cancer Center (MDACC, DiNardo et al. Leukemia 2014). The International Prognostic Scoring System (IPSS) for MDS (Greenberg et al. Blood 1997) discriminated amongst prognostically distinct categories in that cohort, while neither the IPSS for primary myelofibrosis (PMF, Cervantes et al. Blood 2009) nor the revised IPSS (IPSS-R) for MDS (Greenberg et al. Blood 2012) did. Median survival of 21.4 months from the time of diagnosis was reported in a multi-institutional cohort (n=69, Wang et al. Blood 2012). Median survival remained the most powerful predictor.

Methods: Mutational frequencies for the 20 genes tested in all 97 patients were as follows: TET2, 28%; ASXL1, 27%; JAK2, 25%; SRSF2, 22%; EZH2, 15%; SF3B1, 12%; RUNX1, 11%; ZRSR2, 11%; SETBP1, 11%; U2AF1, 11%; NRAS, 10%; DNMT3A, 9%; TP33, 8%; CBL, 4%; ET6V, 4%; NPM1, 4%; IDH2, 2%; KIT, 2%; PHF6, 1% and IDH1, 0%. In addition, the frequency of mutations in ten other genes of interest in hematologic malignancies was assessed: BRAF, 0% (n=52); CSF3R, 4% (n=52); CALR, 4% (n=53); MPL, 3% (n=88); MLL, 1% (n=99); TET2, 8% (n=72); CEBPA, 4% (n=73); KRAS, 4% (n=81); PTEN, 4% (n=82) and FLT3, 2% (n=82). Median survival for the whole cohort (n=97) was 12.4 months (range, 1-173). The 43 MDACC patients in this analysis were included in the cohort of 85 previously reported by DiNardo et al. Median age

Summary/Conclusions: Abnormal karyotype was found in 34.1% of 59 patients at diagnosis and was over-represented in post-PPV MF. No different distribution was detected among genotypes. Abnormal karyotype was associated with lower platelet count, larger splenomegaly, higher circulating blast cells and presence of constitutional symptoms. Concerning outcome, the presence of abnormal karyotype implied inferior survival and, among subtypes, MK remained the most powerful predictor.

References: Aims: We utilized an unbiased screening approach to investigate genome-wide DNA methylation in newly diagnosed JMML patients. We then sought to determine whether a specific DNA methylation signature was capable of predicting outcomes in this heterogeneous disease, with a particular emphasis on identifying a biomarker predictive of spontaneous resolution.

Methods: Genome wide DNA methylation analysis was carried out using the Illumina 450k BeadChip platform in a discovery cohort of 39 well-characterized patients with JMML enriched for those who experienced spontaneous resolution without chemotherapy. A separate cohort of 40 patients with JMML was used for validation. Of note, patients with Noonan syndrome were excluded from both cohorts. All 79 patients were then compared to 22 healthy controls between 1 and 5 years of age using peripheral blood derived DNA.

Results: JMML patients with aggressive disease have a distinctly hypermethylated DNA profile at the most variable CpG sites compared to patients with less aggressive disease as well as healthy controls. Methylation patterns did not differ based on the tissue of origin (peripheral blood, splenic tissue, or bone marrow) and were similar between monocyte enriched cell populations and unsorted mononuclear cells. Unsupervised clustering of the discovery cohort based on the most highly variable CpG sites (top 0.5% ranked by deviation, 15,553 CpG sites) identified three disease clusters. In 19 of 23 patients in the cluster with the lowest levels of methylation, only one patient out of 15 (7%) had an event at 4 years (95% confidence interval [CI], 2-32%). This compared to 45% (5/11) (CI, 17-77%) for patients in the cluster of intermediate levels of methylation and 61% (8/13) (CI, 32-86%) in those patients with the highest level of methylation. The proportion of patients with events differed significantly by cluster (p=0.0039) and remained independently prognostic in multivariable analysis (p=0.033) in the context of age and the number of somatic mutations at diagnosis. We next sought to validate our findings in an independent cohort of 40 patients. We classified each patient in the validation cohort in one of the three clusters defined by the discovery cohort. The proportion of patients having an event at four years was 8% (1/12) (CI, 0-38%) in those with the lowest level of methylation. This compared to 36% (4/11) (CI, 11-69%) for patients with intermediate levels of methylation and 76% (13/17) (CI, 50-93%) for those with the highest levels of methylation. We then compared our combined cohort of 79 JMML patients with 22 healthy, age-appropriate controls. Interestingly, using the same set of CpG sites defined in the discovery cohort, 27/79 JMML patients clustered more closely with the controls than with other patients. Of these 27 patients, 14 (52%)...
LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS: IS NGS PROFILE THE BEST PROGNOSTIC BIOMARKER? V. Geoffroy1,2,*, F. Courtier2, A. Charbonnier 1, E. D’Incan1, C. Sailla1r, B. Mohty1, D. Birmbaum1, M.-J. Mozziaccioni1, A. Murati1, N. Vey1, J. Rey1 1Hematology - Leukemia Department, 2CRCM, 3Biopathology Department, Institut Paoli Calmettes, Marseille, France

Results: Of the 72 patients with AML secondary to MPNs who developed AML, 15.3% (N=11) with azacitidine (AZA) and 38.9% (N=28) with chemotherapy (IC), 7.5-7.7) resulting in an increased hazard ratio of 1.7 (95%CI: 1.2-2.3). The rate of SPM was 12.8 per 1,000 person-years (95%CI: 9.1-17.6) while the rate in the general adult population was 7.6 per 1,000 person-years (95%CI: 7.5-7.7) resulting in an increased hazard ratio of 1.7 (95%CI: 1.2-2.3). The risk for SPM was higher than expected in females (SIR 1.93, 95%CI: 1.2-3.1) while it was not significantly increased in males (SIR 1.11, 95%CI: 0.5-2.2). There was no significant difference between the incidence of SPM in patients with SPM was 14.7% with a median overall survival (OS) from diagnosis to death of 7 months (range: 3-24) respectively. 7 Patients in Complete Cytogenetic Response (CCR) or Acute Leukemia Response-Complete (ALR-C) received an allostet CT as the rate of malignancies may increase over time and reduce life expectancy.

Aims: To assess incidence and outcome of secondary primary malignancies (SPM) in adult mastocytosis patients. Methods: We performed a retrospective analysis of 826 adult (>18 years at diagnosis) mastocytosis patients diagnosed and regularly followed in 6 Italian Institutions. SPM were defined as de novo cancers diagnosed after mastocyto- sis. We excluded from the analysis non-melanoma skin cancers due to the possible under-reporting of such neoplasms by patients themselves. Also, we did not consider newly hematological neoplasms, as they mainly represent a progression from Systemic Mastocytosis (SM) to SM with an Associated Hema- tological Neoplasms (AHN). Standardized Incidence Ratio (SIR) was calculated as the ratio between the observed cases in our cohort and the expected cases in the sex- and age-matched general Italian population in the same time period (these data were retrieved from http://www.registri-tumorit.it). Times to event (patient-years) were calculated from the diagnosis of mastocytosis to the date of SPM diagnosis, death, or last contact, whichever comes first. Survival curves were estimated according to the Kaplan-Meier method.

Results: Males were 450 (54%). Median age at diagnosis was 49.3 years (range 19-84). Median follow-up was 2.3 years (range 0-41). Subtype diagnoses were: Cutaneous Mastocytosis (n=46), Indolent SM (n=633), Smol- dering SM (n=10), SM-AHN (n=54), Aggressive SM (n=47) and Mast cell leukemia (n=2). Fifty-four patients were classified as having mastocytosis in the skin. Overall, 42 patients had a history of malignancies prior to the diagnosis of mastocytosis: in these patients we did not detect any relapse of their prior malignancy after the diagnosis of mastocytosis. A total of 35 SPM were diagnosed in 34 patients (4.1%). Median age at SPM was 56.4 years (range 35-77). Median time from the diagnosis of SPM to SPM was 22 months. The overall rate of SPM was 12.8 per 1,000 person-years (95% CI: 9.1-17.6) while the rate in the general adult population was 7.6 per 1,000 person-years (95% CI: 7.5-7.7) resulting in an increased hazard ratio of 1.7 (95%CI: 1.2-2.3). The risk for SPM was higher than expected in females (SIR 1.93, 95%CI: 1.2-3.1) while it was not significantly increased in males (SIR 1.11, 95%CI: 0.5-2.2). There was no significant difference between the incidence of SPM in patients with SPM was 14.7% with a median overall survival (OS) from diagnosis to death of 7 months (range: 3-24) respectively. 7 Patients in Complete Cytogenetic Response (CCR) or Acute Leukemia Response-Complete (ALR-C) received an allostet CT as the rate of malignancies may increase over time and reduce life expectancy.

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the diagnosis of prePMF have been added (anemia, leukocytosis >11 ×10^9/L, the bone marrow biopsy and an explicit definition of minor clinical criteria for clinical outcomes. For these reasons, standardization of morphologic findings in “true” essential thrombocythemia (ET) as these two entities have different clin-

Methods:

Aims: To explore the clinical course of patients with CSF3R-mutated CNL, and identify risk factor(s) associated with survival.

Results: Data of 47 patients with CSF3R-mutated CNL were collected and analyzed. 35 (76%) patients were male. Median age was 62 years (range, 16-92 years). At diagnosis, 17 (36%) patients had fatigue, 2 (4%) had a fever, and 16 (34%) 16-92 years). At diagnosis, 17 (36%) patients had fatigue, 2 (4%) had a fever, and 16 (34%) patients had constitutional symptoms (98%). We identified in our database all patients affected with ET, prePMF and MPNu. We compared the clinical phenotype at diagnosis and the outcome of ET and MPNu compared to ET, prePMF and MPNu. By comparing clinical phe-

Aims: To compare the clinical phenotype at diagnosis and the outcome of ET and prePMF diagnosed according to 2008 WHO criteria who satisfied these two requirements: a bone marrow fibrosis grade 0-1 at diagnosis and at least one DNA sample to define the mutational status. Firstly, the bone marrow morphol-

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 MPNu and ET is similar.

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Background: The World Health Organization (WHO) classification system for myeloid neoplasms was recently revised in 2016. The revised WHO criteria included an update based on the new diagnostic criteria for pre-MPN (pre-PMF) 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 in ET morphology, and at least one clinical criteria (leukocytosis, anemia, increased LDH, splenomegaly) were classified as prePMF, patients with PMF morphology but without clinical criteria were classified as myeloproliferative neoplasms unclassi-

Results: According to the new criteria our cohort included 269 patients with ET, 109 patients with prePMF and 26 with MPNu. By comparing clinical phe-
CORRELATIONS BETWEEN INFLAMMATORY BIOMARKERS AND INDIVIDUAL SYMPTOMS EXPRESSED BY MYELOFIBROSIS PATIENTS IN THE COMFORT-I TRIAL: ANALYSIS OF BASELINE ASSOCIATIONS AND CHANGES OVER TIME

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

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 (MFSAF 2.0-Mesa JCO 2013; collected during blinded phase of COMFORT-I). Patients were randomized to ruxolitinib vs placebo. BMKs were assessed using Rules-Based Medicine, Inc. (Austin, TX) Human MAP panel. Associations between the individual symptoms measured within the MFSAF and log2-transformed biomarker data were investigated at 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 as fixed effects.

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 Pearson correlation coefficient) with spleen-related symptoms included APOA1, EPO, FERRITIN, MIP1A, and PSAF. Associations with Symptom and Biomarker Change Over the Trial Course. Twenty BMKs were significantly associated with TSS over time. Like at baseline, BMKs appeared to be more often statistically significantly (p<0.05) associated with spleen-related symptoms over time including 25 and 24 BMKs for abdominal discomfort and feeling full, respectively. Night sweats, pain under left ribs, bone or muscle pain, and itchiness were associated with 20, 12, 12, and 9 BMKs each. Strongest associations (p<0.001) between symptoms and BMKs over time included VCA1 (4/6 symptoms+TSS), B2MC (3/6 symptoms+TSS), LEPTIN (3/6 symptoms+TSS), TIMP1 (2/6 symptoms+TSS), TNFRI (2/6 symptoms+TSS), INTLK18 (2/6 symptoms+TSS), and VWD (1/6 symptoms).

Table 1.

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.
Platelet disorders: Basic

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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 activation state, phosphorylation of FAK, and morphological changes were analyzed in transfected cells by WB and immunofluorescence staining.

Results: The patients were 56-year-old Japanese woman and 2 of her 3 sons. They had no bleeding tendencies and near-normal bleeding time (Duke’s method). Hematological examination revealed their decreased platelet counts (58-86 x 10^9/l) with increase of mean platelet volume (12.8-14.5 fL). In all affected family members, giant platelets were observed on the peripheral blood smears. Platelet aggregation induced by ADP (1-10 µmol/l) and collagen (2 µg/ml) was obviously reduced although that induced by ristocetin (1.5 mg/ml) was within normal limit. The family pedigree indicates that the inheritance pattern is autosomal dominant. Common congenital macrothrombocytopenias, such as MYH9 disorders, Bernard-Soulier syndrome and type 2B von Willebrand disease were excluded by the absent leukocyte inclusion bodies, normal ristocetin cofactor induction, and normal von Willebrand factor assays, respectively. FCM revealed that all affected family members had a heterozygous ITGB3 p.T746del mutation. FCM showed decreased surface expression level of αIIbβ3 in the affected member’s platelets. However WB of platelet lysates showed that there was no difference in the total amount of αIIbβ3 among the affected and unaffected members and normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient’s platelets as reflected by the spontaneous binding of PAC-1 antibody. Immunofluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIbβ3-expressing cells and cytoplasmatic localization in αIIbβ3 (p.T746del)-expressing cells. The results indicated 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-Benzetetramine tetrahydrochloride) hindered the localization change of αIIbβ3 and the morphological changes 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.

Summary/Conclusions: The autosomal dominant heterozygous ITGB3 p.T746del mutation was found to be responsible for constitutive activation of αIIbβ3 in the patients’ platelets as well as transfected cells. It is considered that ITGB3 p.T746del mutation unclasps the highly conserved membrane proximal complex of αIIb and β3 cytoplasmatic 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 cytoplasmatic localization. These results suggest that the gain-of-function mutation around membrane region of αIIbβ3 leads to macrothrombocytopenia with impaired surface αIIbβ3 expression.

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CHANGES IN THE GENE EXPRESSION PROFILE OF IMMUNE THROMBOCYTOPENIA PATIENTS TREATED WITH ELTROMBOPAG

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Background: Eltrombopag (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 signal pathways modified before and during the ETP treatment in chronic immune thrombocytopenia (ITP) patients.

Methods: 6 ITP patients (n=14) treated with ETP were evaluated. Complete response (CR) was defined as a platelet count of ≥100 x 10^9/mm^3 and treatment failure was defined as a platelet count of ≤50 x 10^9/mm^3 4 consecutive weeks at the highest recommended dose of ETP, a major bleeding event, or the need to change therapy. RNA was isolated from mononucleated cells pre/post ETP treatment. The “paired” GEP of the ITP patients included the semi-supervised analysis cluster samples before and after (28 day) the treatment with ETP to detect changes attributed to ETP. This paired GEP was showed in Figure 1. The GEP workflow consisted of the following steps: 28-paired samples were hybridized to GeneChip® Human Gene 2.0 ST Array (Affymetrix®). The robust microarray analysis (RMA) algorithm was used for background correction and normalization, while signal expression was calculated by significance analysis of each microarray to provide a robust statistical inference by a permutation method. P-values were provided and adjusted by multiples testing using a false discovery rate (FDR). The pathways and upstream regulators related with the most differentially expressed genes were analyzed by in silico analysis tools: Advaita Bio’s PathwayGuide (http://www.advaitabio.com/pathwayguide) and DAVID Bioinformatics Resources.

Results: The median age of the 14 ITP patients enrolled in the study was 77 years (range, 35-87y), 64% patients (n=9) were treated with ETP after ≥2 lines of treatments. Only 3 patients were splenectomized. Median platelet (P) and white blood cell counts (WBC) increased after treated by ETP at day 28. (P and WBC pre: 14, 15 x 10^9/mm^3 and 6,85 x 10^9/mm^3 vs P and WBC post: 132 x 10^9/mm^3 and 9,1 x 10^9/mm^3). All but two patients achieved CR (85.7%) and other 2 were considered failure of treatment. Regarding the gene expression profile, in silico analysis showed that the expression of 147 genes was modified after ETP treatment; all of them were overexpressed after treatment. Semi-supervised cluster analysis showed 2 groups: pre and post ETP treatment (Figure 1). Pathway analysis revealed that 38 genes were involved in the maintenance of hemostasis, most of them related to platelet activation (PTGS1, GP18A or GP6). Interestingly, the paired GEP pointed out E2F1 and G10IB as possible leaders of the increase of the megakaryopoiesis. Other signaling pathways overexpressed by ETP treatment are downstream routes of PI3K/Akt (G10IB, JAM3, ITGB3 and ITGA2B) and platelet activation (GP6, GP9, GP18A or PTGS1).

Figure 1.

Summary/Conclusions: In ITP patients, ETP can induce overexpression of genes involved in platelet activation and megakaryopoiesis and also alter key/relevant/important-signaling pathways such as JAK/STAT and PI3K/Akt.

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DEFECTIVE PTEF REGULATION CONTRIBUTES TO B CELL HYPERRESPONSIVENESS 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-antigens, specifically immunoglobulin G (IgG)
antibodies against glycoprotein Ib/IX (GPIb/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, low levels 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 signalling 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 autoactive B cells. In this study, we want to determine whether abnormalities in PTEN might contribute to increase B cell responsiveness in this disease and IL-21 mediated PTEN induction was defective. Meanwhile, we want to evaluate the relation between the expression of PTEN in B cells and the prognosis of ITP which will provide a theoretical basis of new treatment strategy for the ITP patient.

**Aims:** PTEN is involved in maintaining normal B cell function. Since B cell overactivity is characteristic of autoimmune 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 age and sex matched health volunteers as 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 rIL-21 CD40L or anti-IGM alone or in combination for 72h and after that the PTEN level was detected by flow cytometry. The proportion and surface activated marker of B cells were determined by flow cytometry.

**Results:** 1. Compared to HC the expression of PTEN was diminished in each CITP B cell population except IgD-CD38low-memory B cells. In addition PTEN 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 positive serum platelet-specific antibody (P=0.03). 3. The capacity of IL-21 to induce PTEN expression in B cells of HC was found by flow cytometry. The proportion and surface activated marker of B cells were determined by flow cytometry.

**Summary/Conclusions:** Immune thrombocytopenia B cell showed decreased levels of PTEN and the decrease was associated with low platelet count and negative serum platelet-specific antibody. The capacity of IL-21 to induce PTEN was defect in CITP. Together, these data suggesting that the defective PTEN expression, regulation and function contribute to B cell hyper-responsiveness in CITP.
tin (cDNA) that bind to galactose, N-acetylatedamino 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 RCA binding compared to healthy donors (median fold increase (FI): 1.21, range: 1.08 - 1.40). 9/37 sera induced higher ECL binding (median FI: 1.02, range: 1.08 - 1.15). In contrast, 8/37 sera showed strong decrease in RCA binding (median FI: 0.52, range: 0.50 - 0.59). Sera from healthy donors did not induced significant change. Interestingly, not only GP-Ib/IX-AAbs but also GP-IIb/IIIa AAbs were able to modify glycan pattern. In NOD/SCID mice the administration of AAbs induced an accelerated clearance of human PLTs from the circulation. The destruction of human PLTs by ITP-AAbs was decreased but not completely prevented by a specific neuraminidase inhibitor that blocks glycan changes on PLT surface (survival of human PLTs after 5h: 48%, range 41-53% vs 29%, range: 22-33%). Summary/Conclusions: Our results demonstrate that AAbs from ITP patients are able to induce cleavage of glycan moieties on the PLT surface in distinct manners. Antibody-modified modification of glycan patterns seems to contribute to AAb-mediated PLT destruction. P365 NOVEL RUNX1 MUTATIONS IN FAMILIES WITH INHERITED THROMBOCYTOPENIC P. Noris1,1, D. De Rocco2, F. Melazzini1, C. Marconii3, A. Pecci1, R. Bottega2, C. Gnan1, F. Palombo2, P. Giordano2, M.S. Coccioli3, A.C. Glembotsky4, E. Cigliani1, P.G. Heller5, M. Seri1, A. Savoia1

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Background: Familial platelet disorder with propensity to acute myeloid leukemia (FPD/AML) is a rare autosomal dominant inherited thrombocytopenia (IT) caused by mutations in the hematopoietic transcription factor RUNX1; an important hallmark of this IT is the increased risk of developing myeloid neoplasms, such as AML and myelodysplastic syndromes (MDS). FPD/AML is caused by different mutations of RUNX1 encoding the DNA binding subunit (known as core binding factor-alpha, CBF-alpha) of the CBF transcription complex. The N-terminus domain of RUNX1 (runt-homologous domain) mediates DNA binding and heterodimerization to CBF-beta, the other subunit of the CBF complex. The C-terminus of RUNX1 includes domains that are involved in transcription activation and repression. This IT is characterized by impaired megakaryopoiesis and moderate thrombocytopenia, with normal-sized and dysfunctional platelets.

Aims: To unravel the molecular basis of ITs and to improve our knowledge on the molecular basis and clinical-laboratory picture of FPD/AML.

Methods: Whole exome sequencing (WES) was performed in 86 propositi with an unknown IT after the diagnostic workup based on the most updated diagnostic algorithm for ITs (Clin Genet 2016;89:141). RUNX1 variants detected by WES were confirmed by Sanger sequencing in the propositi and all available family members, which also undergo clinical-laboratory characterization. The study was approved by the Institutional Review Board of the IRCCS Policlinico S. Matteo Foundation; all patients gave written informed consent.

Results: We identified three pedigrees (families 1-3) with different RUNX1 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 RUNX1 mutations had mild thrombocytopenia (platelet count ranging from 70 to 130 x 10^9/L) with mild bleeding tendency. Platelet sizes were within the normal range in all the six patients analyzed, and the serum level of thrombopoietin was normal or moderately increased. No specific morphological alteration of platelets was detected, except for moderate reduction in the alpha-granule content in family 1, confirmed by immunofluorescence analysis. The surface expression of the major platelet glycoprotein (GP) complexes GPIb-IIIa and GPIb-IX-V was normal. In family 1 a moderate reduction of GPla was detected, regardless of genotypes at the ITGA2 locus. A defective aggregation was detected after platelet stimulation with collagen 4 mcg/ml and ADP 2 mcM in the five patients investigated; normal responses were obtained using collagen 20 mcg/ml, ADP 20 mcM and ristocetin 1.5 mg/mL, suggesting mild functional platelet defects. Of note, three patients 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 J. Grainger1, J. Busse2, N. Cooper3, M. Tarantilino4, V. Blanchette5, J. Despotovic6, A. Masch8, N. Carpenter9, M. Eisen10, B. Mehta11

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Background: The use of romiplostim in children with ITP has been evaluated in phase 1/2 and 3 studies. Here we describe children with ITP who will receive open-label SC romiplostim for up to 3 years (y).

Aims: To assess platelet responses in children with ITP receiving romiplostim.

Methods: Eligible children, recruited in 16 countries worldwide, had ITP for ≥6 months, ≥1 prior ITP therapy, and platelet (plt) counts ≤30×10^9/L. Weekly SC dosing started at 1 μg/kg and was titrated in 1 μg/kg increments up to 10 μg/kg to achieve plt counts of 50-200×10^9/L. The primary endpoint was the % of time in the first 6 months with a plt response (plt count ≥50×10^9/L without rescue medication use in the past 4 weeks).

Results: As of 15 Mar 2016, 145 patients received ≥1 dose. At baseline, medi- an (min-max) age was 10 (2-17) y; 51% were female; 4% had prior splenectomy. Median (min-max) ITP duration was 1.9 (0.5-12.3) y and plt count was 13 (2-168)×10^9/L. The median (Q1, Q3)% of time with a plt response in the

Figure 1.
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 ≥20x10^9/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 thrombolysis dose 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 epis-taxis (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, hematemesis, 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 base-line bone marrow biopsies (bone marrow biopsies were obtained at European sites), all had modified Bauermeister scores of grade 0 (no reticulin) or 1 (fine fibers) and bone marrows typical for ITP. Of these 30 patients, 21 had evaluable on-study biopsies obtained after ~1 year of treatment, with no increases in 2 or more grades, findings of collagen, or bone marrow abnormalities (Figure 1).

**Summary/Conclusions:**
In the first 1-2 years 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 sig-nals. No effects of romiplostim were observed on the bone marrow in the subset of patients who had marrow biopsies. Future datasets for years 2 and 3 in this study, the largest of romiplostim in children with ITP with 97 patient-years of exposure to date, will provide more information on platelet response, dose requirements, and safety.

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**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 thiopyridine class of anti-platelet drugs, of which clopidogrel and prasugrel are the most well known, target the P2Y12 receptor on platelets, blocking the effects of the platelet agonist ADP. However, the effect of these drugs is variable among 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 thiopyridine derivatives synthesized by our group through examining their potential as in-vitro inhibitors of platelet function.

**Methods:** Healthy human platelets were isolated and incubated with novel thiopyridine compounds (D.J0081, D.J0199, D.J0201, D.J0206, D.J0171, DJ0097) (10µM, 30min) prior to stimulation with ADP (10µM) and analysis of alpha granule secretion (CD62P expression), GP Ib/IIa activation (PA1C expression) and platelet leukocyte aggregate (PLA) formation using flow cytometry. Furthermore, light transmission aggregometry (LTA) was used to assess ADP induced platelet aggregation after these treatments. As clopidogrel is usually prescribed in combination with the COX-1 inhibitor acetylsalicylic acid (ASA), synergy of the novel compounds with ASA (30µM) was also analysed by LTA. All results were compared to ADP-stimulated samples and samples treated with clopidogrel (10µM, 30min) prior to ADP stimulation.

**Results:** All six novel compounds demonstrated a significant reduction in ADP-mediated platelet aggregation (P<0.001), CD62P expression (p<0.001), PAC1 expression (p<0.01) and PLA formation (p<0.05). These compounds were also shown to enhance the inhibitory effects of ASA. DJ0171 and DJ0199 were particularly potent, displaying greater inhibitory effect than clopidogrel.

**Summary/Conclusions:** The study demonstrates the potential for new thiopyridine compounds as modulators of platelet function and points to the possibility of future use in patients at risk of platelet hyperactivity and thrombosis.
ETIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA AND SURVIVAL FOLLOWING RELAPSE AFTER HLA IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (aHCT) 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 aHCT provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

Aims: To estimate HRU and costs among newly-diagnosed AML patients (pts) in a US commercially insured population by receipt of chemotherapy (CT) or another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post aHCT provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

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; another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post aHCT provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

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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; another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post aHCT provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.
AL amyloidosis may be greater for those with PN involvement non-AL-PN patients (p <0.05 for all). These significant differences also exceed significant differences in SF-36v2 scores among AL-PN patients as compared to non-AL-PN patients, even after controlling for other types of organ involvement. Nearly all AL-PN patients (97.6%) reported multi-system involvement.

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 19.1 mos in untreated pts. Mean total costs were higher for treated pts ($386,711 vs untreated pts ($83,274). In treated pts, total mean 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).

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Summary/Conclusions: HRU and costs of managing AML pts are considerable, with greatest HRU and cost in pts receiving CT or SCT.

P373

HEALTH-RELATED QUALITY OF LIFE IN AL AMYLOIDOSIS PATIENTS WITH NERVOUS SYSTEM INVOLVEMENT

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Background: In light chain (AL) amyloidosis, misfolded light chains accumulate and cause progressive peripheral neuropathy (PN) and failure of critical organs such as the heart and kidneys. Consequently, a progressive, ascending seniormotor neuropathy is often a related clinical finding.

Aims: This study describes disease characteristics and health-related quality of life (HRQoL) in AL amyloidosis patients with peripheral nerve involvement (AL-PN).

Methods: An online survey was administered to AL-PN (n=126) and non-nerve–affected (n=215) patients to assess patient characteristics and HRQoL (based on the SF-36v2 Health Survey [SF-36v2]). The survey measures eight health concepts – physical function, role physical (RP), 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 (HRQoL) in AL amyloidosis patients with peripheral nerve involvement (AL-PN).

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 19.1 mos in untreated pts. Mean total costs were higher for treated pts ($386,711 vs untreated pts ($83,274). In treated pts, total mean 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).

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Summary/Conclusions: This study suggests that the burden of illness from AL amyloidosis may be greater for those with PN involvement versus those without. AL-PN patients also experienced more complicated journeys to diagnosis and significantly worse symptoms related to nervous systems and physical HRQoL. The SF36v2, a reliable and valid assessment of HRQoL in AL amyloidosis studies, was sensitive to differences in HRQoL between AL-PN and non-AL-PN patients. Future research should examine whether improvements in neuropathy symptoms following treatment subsequently lead to improvements in HRQoL among patients with AL-PN. These findings are helpful for patient-focused drug development and supportive treatments.

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ACCESS TO COMMUNITY CHEMOTHERAPY IMPROVES PATIENT QUALITY OF LIFE

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1Faculty of Life Sciences and Education, University of South Wales, Pontypridd, 2Tenovus, 3Haematology, University Hospital of Wales, Cardiff, United Kingdom

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.

Target group was patients with myeloma, aiming for up to 20 a day once or twice a week.

Methods: The first drugs administered on the Mobile Unit were zoledronate infusions, followed by bortezomib. When twice weekly doses were required, patients collected an additional injection pack which they could self-administer in their homes, thereby saving another trip to hospital. Any criticisms received focused on the locations we chose to site the Mobile Unit in relation to accessibility via public transport.

Summary/Conclusions: Treatment in the community alleviates the stress of treatment and with minimal waiting times it gives some patients the ability to maintain family life and where possible to continue to work. It is both feasible and acceptable to begin to ambulate many different sorts of treatments. The possibilities opening up for haematology include rituximab maintenance; community blood transfusions; delivering pentamidine for patients at risk of pneumocystis infection; late effects clinics for teenage and young adult cancer patients; and myeloproliferative neoplasm clinics, possibly near community pharmacies to facilitate dispensing medicines such as hydroxyurea.
which will have a substantial price difference compared with nilotinib. However, given the possible changes in switching of TFR, this price difference may not translate into a similar magnitude of difference in drug budget for first-line nilotinib vs imatinib due to better MR with nilotinib.

**Aims:** To estimate the budget impact for first-line nilotinib vs imatinib when considering generic imatinib pricing, early treatment-switching, and TFR.

**Methods:** This was a state transition model implemented in Microsoft Excel. The GAH model was used to predict 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 MR. Duration of first-line or second-line TFR was based on an extrapolation of ENESTnd trial. Total treatment costs 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 368 at 60 mos.); achieved MR4.5 on first-line therapy (347 vs 183 by 60 mos.); entered TFR on either first- or second-line (494 vs 400 by 60 mos.); and was in any TFR at 60 months (293 vs 200). The incremental budget impact per patient for first-line nilotinib vs imatinib decreased each year from €16,482 in Year 1 to €377 in Year 5. Overall, the 64% lower drug acquisition costs per month of imatinib ($1,063) vs nilotinib ($2,952) provided only a 17% lower total budget impact over five years ($141,204 vs $170,002) per patient.

**Summary/Conclusions:** Results from the model considered more switching as per 2013 ELN guidelines, which resulted in greater and quicker switching than observed in ENESTnd. Overall, it was projected that imatinib patients who receive first-line nilotinib would have earlier and more sustained molecular response requirements for TFR eligibility—and be subject to less treatment-switching. The model projected that less than 50% of patients would remain on first-line imatinib at 15 months. This would significantly reduce the drug budget 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. Bonanadi,1,2 R. Gonzalez,1 A. Cruz-Pentaf1, L. Garcia Iglesias,1 I. Jauregui1, E. Pérez-Persona,1 S. R. Lluch,1 C. Marrero,1 M. Zudaire1,2 M. Gironella1,2,3, R. Fernández-Ordoño1,4, M. Arnán1,5, C. Olivier6, C. Encinas2, J.A. Soler7, Á. Ramírez Payer8,9, P. Fernández10, D. Vilanova11, J. de la Rubia12,13,14.

**References:**

1Department of Hematology, H. U. La Fe, Valencia, 2Department of Hematology, H. U. of Canarias, Santa Cruz de Tenerife, 3Servicio de Geriatria, Hospital Universitario La Princesa, 4Department of Clinical Research and Epidemiology, Centro Médico IMI, Madrid, 5Department of Hematology, Hospital de Canarias, 6Department of Hematology, R. de Aricuina, Arica, 7Hematology Department, H. U. de la Ribera, Valencia, 8Hematology Department, H. U. Nuestra Señora de la Candelaria, Santa Cruz de Tenerife, 9Hematology Department, C.H. de Navarra, Pamplona, 10Hematology Department, H. U. Vall d’Hebron, Barcelona, 11Hematology Department, H. U. Infantil Leonor, Madrid, 12Hematology Department, IDIBELL, H. Dúran i Reynals, Barcelona, 13Hematology Department, C.H. de Segovia, Segovia, 14Hematology Department, H. U. Gregorio Marañón, Madrid, 15Hematology Department, C.S. Parc Taulí, Barcelona, 16Hematology Department, H. U. Central de Asturias, Oviedo, 17Hematology Department, H. U. de Lérida, 18Hematology Department, H. U. de Salamanca, Spain.

**Background:** The Geriatric Assessment in Hematology (GAH) scale is a newly developed tool that is intended to be an ancillary questionnaire to better categorize elderly patients diagnosed with comorbidities that would impact the need for intensive treatment in routine clinical practice. It is a brief (<12 min) and easy instrument, which takes into account 8 dimensions of geriatric assessment that are predicted to substantially offset the lower unit cost for generic imatinib.

**Aims:** To determine the weights for each dimension of the GAH scale and the cutoff 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:** This was an observational, 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.

**Results:** A total of 108 patients (women, 53.7%; median age [IQR], 78 [73-83] years) out of 360 included in the main study were evaluated. During treatment administration, 61 (56.5%) patients developed treatment-related toxicities, requiring discontinuation/modification of the initial therapy. The coefficients for the dimensions are -7 for number of drugs, -10 for gait speed, 2 for mood, 27 for nutritional deficiencies (e.g., hypocholesterolemia, hypoalbuminemia, weight loss). Diets rich in fruits, vegetables, legumes, whole grains, fish, nuts, and low-fat dairy products are associated with a decrease in inflammatory (e.g., TNF-a, IL-6, and CRP) and thrombotic markers (e.g., homocysteine, fibrinogen; Chrysosouhou 2004, Smidowicz 2015). To date, no studies have evaluated the nutritional needs or preferences of MPN patients in regards to dietary advice.

**Aims:** The aim of this project was to determine the nutritional needs and preferences that will help inform the creation of a tailored MPN dietary intervention.

**Methods:** An internet-based survey was hosted by the Mayo Clinic Survey Center and promoted on multiple MPN-based forums, Facebook pages, and websites during February of 2017. The survey included data on demographics, MPN characteristics, nutritional habits, supplement use, and symptom burden using the MN-SP SFSS/MPN-10 (Emanuel 2012).

**Results:** Demographics and symptom burden: 919 international MPN patients participated in the survey, and to the online survey, 22% were diagnosed with MF, 37.1% with PV, and 37.4% with ET. Respondents represented MPN patients from 37 countries, although the highest proportion of respondents were from the United States (48.8%), United Kingdom (32.7%), Australia (6%), and Canada (3.6%). Average MPN-SP SFSS T score was 33.6 (SD=17). Dietary Habits: 22.5% of patients described having changed their strategies or that they maintained their weight-loss strategies (e.g., hypocholesterolemia, weight loss). Diet rich in fruits, vegetables, legumes, whole grains, fish, nuts, and low-fat dairy products are associated with a decrease in inflammatory (e.g., TNF-a, IL-6, and CRP) and thrombotic markers (e.g., homocysteine, fibrinogen; Chrysosouhou 2004, Smidowicz 2015). To date, no studies have evaluated the nutritional needs or preferences of MPN patients in regards to dietary advice.

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**Summary/Conclusions:** Results from the model considered more switching as per 2013 ELN guidelines, which resulted in greater and quicker switching than observed in ENESTnd. Overall, it was projected that imatinib patients who receive first-line nilotinib would have earlier and more sustained molecular response requirements for TFR eligibility—and be subject to less treatment-switching. The model projected that less than 50% of patients would remain on first-line imatinib at 15 months. This would significantly reduce the drug budget 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.
using diet to help control their symptoms or MPN disease. Patients most often utilized books (28.2%), websites (27.1%), health care providers such as physicians, NPs or naturopaths (28.2%), online forums (23.2%), friends (12.2%), nutritionists (9.5%), phone or tablet applications (9.1%), or videos (4.2%) for nutritional education. The vast majority (95.9%) of MPN patients endorsed being willing to eat only certain foods if it helped to control symptom burden and or could help their MPN to stabilize or reduce the risk of their MPN getting worse (98.0%).

Table 1.

Summary/Conclusions: There remains an unmet need for symptom burden improvement in low-risk MPN patients or among those who have reoccurrence of symptoms while on JAK inhibitor therapy. Nutritional interventions for MPN patients have not previously been investigated and have the potential to be paired with traditional interventions to allow MPN patients to self-manage symptom burden. This study represents the first evaluation of MPN-related nutritional habits and preferences. These results will be used to inform the creation of an MPN nutritional intervention with the goal of improving symptom burden and reducing inflammation.

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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=18/30, 50%), taking into account patients’ preferences (n=10/30, 33.3%). Most physicians felt uncomfortable delivering bad news such as initial diagnosis, relapse and poor prognosis (n=25/30, 83.3%). Self-reported needs included (i) communication skills training (n=20/30, 66.7%); (ii) psychological support (n=7/30, 23.3%); and, (iii) working in a multidisciplinary team (n=8/30, 26.7%).

Summary/Conclusions: In the absence of structured communication guidance there is great uncertainty among physicians concerning their skills on communicating CLL nature and handling difficult situations, leading to distress endangering their engagement in a healthy relationship with the patient. Additional studies are warranted at European level for identifying physician needs in different countries aiming at improving their communication skills to support and empower CLL patients for participating in their own care and enhance their QoL.
OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE LEUKEMIA ABOVE 70 YEARS OF AGE: ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMRT

O. Ringdén, A. Boumendil, M. Labopin, B. Sadeghi, B. Savani, M. Mohy, A. Nagler.

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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 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 of age with that of younger patients.

Methods: AML patients aged between 50 and 90 years old receiving a first or second allo SCT between 2004 and 2014 with MSD or UD donor were included in the study. Comparison of outcomes of patients aged above 70 with that of patients between 50-70 years were performed for the whole group and 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 of age (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 stem cell grafts (96 vs 91%, p<0.001), more often unrelated donors (79% vs 59%, p<0.001) and poorer Karnofsky score (36% below 90, p<0.001), received more often reduced intensity conditioning (80 vs 63%, p<0.001). Incidence of acute GVHD III/V, chronic GVHD and relapse were the same in the two groups in multivariate analysis. Non-relapse mortality (NRM) at two years was 34% (95% CI 31–38%) in pts above and 24% (25%–32%) in those below 70 years of age (p<0.001). Overall survival and leukemia-free survival (LFS) at 2 years was 38% (95% CI 34–42) vs 50% (95% CI 49–50) p=0.001 and 33% (95% CI 29–37) vs 44% (95% CI 43-45) in the two groups, respectively (p<0.001). Among pts in CR1, 2 years survival was 43% (95% CI 37–51) vs 57% (95% CI 56–58) (p=0.001), in CR2 it was 36% (95% CI 27–27) vs 52% (95% CI 50–54) (p=0.002) and in advanced disease 35% (95% CI 29-41) vs 33% (95% CI 31-34) (p=0.36) in pts above and below 70 years of age, respectively. Among pts older than 70 years of age a Karnofsky score >80% was associated with improved survival and LFS in multivariate analysis (HR 0.7 95%CI 0.5-0.9, p=0.005 and HR 0.7 95%CI 0.5-0.9 , p=0.003 respectively).

Summary/Conclusions: In AML with CR1, CR2 status at allo SCT, pts above 70 years of age have worse NRM, survival and LFS compared to pts 50-70 years of age. In pts above 70 years of age Karnofsky score is of significant importance for outcome.

OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE LEUKEMIA ABOVE 70 YEARS OF AGE: ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMRT

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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.
Sickle Cell Disease (SCD) and Diamond-Blackfan Anemia (DBA). Bertaina et al (Blood, 2014) have previously shown that abTCR depleted haplo-transplantation in children with multiple types of non-malignant disorders was feasible. An ongoing Phase II trial evaluates the safety and efficacy of post-transplant infusion of donor T-cells transduced with the iC9 suicide gene (BPX-501) cells. (ClinicalTrials.gov identifier: NCT02055869). The iC9 vector contains the sequence for the CD19 marker, so that the BPX-501 cells (CD3+CD19-) can be tracked in peripheral blood. We report on 15 children with hemoglobinopathies and ED.

Aims: This study was performed to determine the clinical impact of infusing BPX-501 T cells post alβ 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, thiopeta 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/ββ, 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 was achieved in a median of 23.5 days (range 14-55) and there were two patients re-hospitalized at 30, 163 days respectively. Grade III 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 days), 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 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.

Summary/Conclusions: These data suggest that Haplo-HSCT combined with infusion of BPX-501 T cells with a suicide gene may be a safe and curative option for children with hemoglobinopathies and ED who lack a matched donor. Infusion of gene modified T cells with an inducible suicide mechanism, combined with selective αβ T-cell depletion, offers the potential to rapidly reverse GVHD and eliminate the need for the use of GVHD prophylaxis. Additionally, this approach results in rapid hematopoietic and immune reconstitution for Haplo-HSCT recipients.

EXCELLENT RESPONSE, LOW TRM AND GOOD SURVIVAL IN PATIENTS WITH THERAPY-REFRACTORY aGVHD AFTER TREATMENT WITH EQUIPOTENT MSCS OF A SERUM-FREE MSC-BANK GENERATED FROM POOLED BM-MNCs OF MULTIPLE DONORS

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Background: Higher peak tacrolimus concentrations after allogeneic transplantation increase the risk of endothelial cell damage and complications.

P383 HIGHER PEAK TACROLISMUS CONCENTRATIONS AFTER ALLOGENEIC TRANSPLANTATION INCREASE THE RISK OF ENDOTHELIAL CELL DAMAGE AND COMPLICATIONS

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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-process-1 vials from which end-of-process-2 MSC products are expanded for clinical use. The manufacturing process is fully GMP-compliant and generates an animal-serum and antibiotic-free MSC 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 alloantigen-driven reaction in mixed lymphocyte reactions (Kuci et al. Haematologica 2016. 101 (8): 885-894).

Aims: A “hospital exemption” issued by the national regulatory authority Pau-Ehrich-Institute (Number: PEI. A11748.01.1) licenses the clinical use of these products for patients with steroid refractory GVHD. On the basis of this licence patients were with severe GVHD were treated who were either non responsive or refractory to at least two lines of immunosuppressive therapy after treatment with steroids for at least 7 days.

Methods: Using these standardized MSC products altogether 52 patients were treated between December 2014 and December 2016. Patients were male (n=31, 60%) or female (n=21, 40%) and were transplanted for leukemia (n=38, 73%), or non-malignant (n=14, 27%) diseases. Median age was 8 (range: 0.5-54) years. All patients received Stem cell source and ATG (n=17, 33%), 6MMP (n=19, 38%) or MMF (n=19, 38%) and derived from BM (n=10, 19%) and deriving from blood (n=24, 46%) or cord blood (n=1, 2%). Patients were suffering from aGVHD grade II (n=3, 5.5%), III (n=14, 27%) or IV (n=31, 60%) or extensive cGVHD (n=4, 7.5%). Acute GVHD occurred at a median of 52 days (5-260 days) after transplant. Patients received in weekly intervals up to four MSC infusions after having failed to respond to the treatment with either two lines (n=10, 19%), three lines (n=20, 38%), four lines (n=10, 19%), 5 lines (n=7, 13%), six lines (n=4, 8%), or 7 lines (n=1, 2%) of immune suppressive drugs.

Results: Response was defined as either complete response (CR) in patients with a complete or full resolution of all signs of GVHD, partial response (PR) in patients who showed one overall GVHD grade less according to the Glucksberg criteria, or non response (NR) at day 28 after first MSC transfusion. At day +28, 12 patients (23%) achieved CR, 29 patients (57%) PR (overall response 80%), 8 patients (17%) NR, and in 2 patients (4%) no data were available at day +28. At the last follow up of GVHD, 29 patients (56%) were in CR, 13 patents (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±5%, cumulative relapse incidence (CIR) of 14±4%, and a 2 years overall survival (OS) of 65±6%. Patients with aGVHD III and IV had an OS survival probability at 2 years of 77±12% and 59±35%, respectively but in patients with severe acute GVHD. There was no difference between younger (n=40) and older patients (n=12) than 16 years.

Summary/Conclusions: Treatment with standardized equipotent MSCs from the “FRANKFURT MSC-BANK” offers an excellent chance to overcome treatment-resistant and steroid-refractory acute GVHD.
and pts with unavailable TAC concentration data were excluded. A total of 253 pts was eligible. All pts received standard GVHD prophylaxis by continuous intravenous (iv) TAC with starting dose of 0.02 mg/kg/day from 1 day before allo-HSCT (day -1) and iv methotrexate on day 1, 3, 6 at dose of 10 mg/m², 7mg/m², respectively. TAC dosage was adjusted to target the serum concentration of 8-12 ng/ml until at least day 30 and then tapered. TAC was rapidly tapered in case of the pathological diagnosis of TAM. TAC serum concentration was sequentially examined tri weekly until day 35 at least. The primary endpoint of this study was to evaluate the cumulative incidence of TRC-EC in relation to weekly mean/peak TAC concentration. Secondary endpoint was OS.

Results: Median patient age was 45 years (16-68). The risks of disease were standard in 168 and high in 89 pts. Forty pts were diagnosed of TRC-EC: 5OS: 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=0.03), and pts with unavailable TAC concentration data were excluded. A total of 253 pts (P=0.018) and grade 3-4 acute GVHD (P<0.01) were significantly associated with the development of TRC-EC. Higher mean TAC concentrations (MTC) during day 0-7 was correlated with higher incidence of TRC-EC, but not significant (P=0.069). In multivariate Fine-Gray analysis, high PTC during day 22-28 (HR: 1.92, 95%CI, 1.07-3.45, P=0.026) and grade 4-acute GVHD (HR: 8.33, 95%CI, 4.18-16.59, P<0.01) remained associated with TRC-EC occurrence. The probability of OS at 15-months was 0.56 (95%CI, 0.47-0.64). Univariate analysis showed that pts diagnosed TRC-EC (P<0.01), pts older than 50 (P<0.01), pts with high risk disease (P<0.01) and pts who received reduced intensity conditioning regimen (P=0.01) were significantly associated with poor OS. PTC and MTC at any time-period were not significant factors for OS. By Cox proportion-al-hazards regression models, TRC-EC diagnosis (HR: 1.90, 95%CI, 1.16-3.11, P=0.011) and high disease risk at transplant (HR: 1.76, 95%CI, 1.14-2.73, P=0.011) were significantly associated with poor OS (Figure 1).

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.

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IMPACT OF CONDITIONING REGIMEN ON OUTCOMES OF T-REPLETE HAPLOIDENTICAL TRANSPLANTATION FOR PATIENTS OVER 45 YEARS-OLD WITH AML: A STUDY ON BEHALF OF THE ACUTE LEUKEMIA-LYMPHOMA: A DECISION ANALYSIS

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Background: T-cell replete haplo-identical stem cell transplantation (haplo-SCT) is a valid therapeutic option for adult patients (pts) with high risk acute myeloid leukemia (AML) lacking a sibling or unrelated donor. However the impact of reduced intensity (RIC) vs myeloablative (MAC) conditioning regi-

men is not conclusive as no randomized study addressing this question is yet available.

Aims: In the present study we compared the outcome of RIC and MAC in pts with AML older than 45 yrs undergoing haploSCT. The aim of the study was to confirm the efficacy and feasibility of RIC among a population for which the choice of conditioning intensity is more related to center strategy than pts comorbidities or disease status.

Methods: We retrospectively compared the outcomes of 614 pts with de novo or secondary AML transplanted between 2007 and 2015 from an haplo-identical donor using either RIC (n=365) or MAC (n=249) regimens. Age was categorized in three subgroups (45-55 yrs, 55-60 yrs, >60 yrs). Patients receiving a previous allogeneic transplantation were excluded. RIC was defined according to EBMT definitions.

Results: The median follow up for MAC and RIC was 24 and 20 months, respectively and the median year of transplant was 2013 for both. Pts receiving a RIC were older (55 yrs in MAC vs 61 yrs in RIC, p<10-4). Secondary AML was more frequent in RIC vs MAC (31% vs 22%) while 77% of MAC and 68% of RIC were transplant for de novo AML, p<0.01. No differences were found on disease status and Karnofsky performance status (KPS) at transplant: pts were in CR1 (MAC: 44%, RIC: 40.5%), CR2/3 (MAC: 17%; RIC: 17%) or had active disease (MAC: 40%; RIC: 43%), p<0.08; 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 m1nTBF (27%) and low dose TBI+Fludarabine (24%). RIC regimen was more frequently associated with multi-organ involvement as stem cell source (MAC 42% vs RIC 55%, p<0.002). Post-transplant cyclophos-phamide 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 II-IV 24% vs 31% (p=0.05), and cGVHD 27% vs 26% vs 39% (p=0.17), OS 46% vs 39% (p=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 fol-

lowed by disease recurrence. In multivariate analysis, the type of conditioning regimen was not associated with risk of relapse or treatment failure: RI (HR: 1.22, p=0.28), NRM (HR: 0.92, p=0.63), acute GVHD grade II-IV (HR: 1.14, p=0.48), chronic GVHD (HR: 1.26, p=0.30), LFS (HR: 1.03, p=0.77), GRFS (HR: 1.07, p=0.55), OS (HR: 1.05, p=0.68). Disease status was associated with outcomes (active disease vs CR): RI (HR: 2.44, p<10-4), LFS (HR: 1.75, p<10-4), GRFS (HR: 1.72, p<10-4), OS (HR: 1.71, p<10-4) as well as KPS>90: NRM (HR: 0.53, p=0.0002), LFS (HR: 0.67, p=0.001), GRFS (HR: 0.74, p=0.014), OS (HR: 0.62, p=0.0002).

Summary/Conclusions: In our study no differences were found between RIC and MAC regimens for haplo-SCT in adults with AML including the age stratified populations. Disease status and performance status were the major predictors of transplantation outcome, while conditioning intensity had no effect. These results may serve as the background for a well design randomized study com-

paring RIC vs MAC for haplo-SCT in adult pts with AML.

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ROLE OF UPFRONT ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH AGGRESSIVE ADULT T-CELL LEUKEMIA-LYMPHOMA: A DECISION ANALYSIS

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logy, Oita University Faculty of Medicine, Oita, 10Department of Hematology, National Hospital Organization Kumamoto Medical Center, Kumamoto, 11Division of Biostatistics, Tohoku University Graduate School of Medicine, Sendai, Japan.

Background: Patients with aggressive adult T cell leukemia-lymphoma (ATL) are considered for therapy that may offer cure. However, there is still controversy regarding the indication of up-front allogeneic hematopoietic stem cell transplantation (allo-HSCT) as no prospective randomized controlled trial (RCT) has been conducted due to a rarity of patients with ATL even in Japan.
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-H SCT 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 from 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.

**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.

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OUTCOMES OF THIOTEPA BASED REDUCED-INTENSITY CONDITIONING VERSUS STANDARD-REDUCED-INTENSITY CONDITIONING IN ADULT PATIENTS UNDERGOING DOUBLE-UNIT CORD-BLOOD HEMATOPOIETIC STEM CELL TRANSPLANTATION

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**Background:** Cord blood transplantation (CBT) is an established alternative source for hematopoietic stem-cells in patients without matched donor. However, the most commonly used high-dose total-body-irradiation (TBI) myeloablative conditioning (MAC) results in high treatment related mortality (TRM). Non-myeloablative and reduced-intensity conditioning (RIC) have been studied to decrease TRM and provide curative chance to the elderly and those with comorbidities. However, these strategies are associated with higher relapse-rate and graft rejection. A novel-RIC using addition of thiotepa and higher dose of TBI to standard RIC has shown to result in sustained donor engraftment. Our study compares transplant-related-outcomes in patients who underwent first double-unit CBT with standard-RIC regimen of fludarabine (Flu, 200mg/m2), cyclophosphamide (Cy, 50mg/kg), and TBI (200Cy or 300CyGy) versus this standard-RIC regimen with addition of thiotepa (10mg/kg) and increased dose of TBI (50CyGy).

**Aims:** 1. To compare transplant related outcomes in CBT recipients who received standard-RIC (FluCyTBI) to those who received novel-RIC (FluCy with addition of thiotepa and increased dose of TBI). 2. To identify optimal conditioning regimen in patients undergoing UCT.

**Methods:** After IRB approval, consecutive patients undergoing CBT from 08/2009 to 08/2016 were evaluated and data retrospectively abstracted. Patient selection, graft-versus-host disease prophylaxis and transfusions were per institutional standards and conditioning regimens were compared as described. Results: 27 of the 99 patients who underwent autologeneic 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%) patients (grade 2-4: n=15, 29%; grade 3-4: n=5, 10% in standard-RIC group and in 32 (66%) patients (grade 2-4: n=29, 62%; grade 3-4: n=5, 11%) in novel-RIC group. Chronic-GVHD (cGVHD) occurred in 18 patients (n=7, 14% in standard-RIC; n=11, 23% in novel-RIC group). The one-year cumulative incidence of relapse was 36% (n=15) in standard-RIC while it was 15% (n=5) in novel-RIC cohort. Median relapse free survival (RFS) was significantly improved in novel-RIC cohort compared to standard-RIC (HR, 0.32, CI:0.11-0.76, p=0.01). Median RFS was 29 months in standard-RIC cohort while median RFS was not reached in novel-RIC cohort. The one-year cumulative incidence of transplant related mortality (TRM) was 22% (n=10) in those who received standard-RIC while it was 16% (n=7) in those who received novel-RIC. TRM was not significantly different between the standard-RIC and novel-RIC cohorts. Median follow-up in standard-RCI cohort was 9.3 months (range, 0.16-79) and 13 months (range, 1.4-38) in novel-RCI cohort. The overall survival (OS) was significantly better in novel-RCI cohort compared to standard-RCI (HR 0.49, CI: 0.25-0.94, p=0.03). Median OS was 17 months in standard-RIC cohort while median OS was not reached in novel-RIC group (Figure 1).

**Summary/Conclusions:** In our study, RIC consisting of FluCy with addition of thiotepa and increased dose of TBI in patients undergoing double-cord UCT was associated with improved OS and improved RFS without increase in TRM as compared to standard RIC. While older and more comorbid patients might experience increased TRM with the thiotepa based regimen, these data suggest that consideration of this regimen may be appropriate in fit, older patients.

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INTERFERON-Α 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 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:** Determined the efficacy of MRD-directed 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 (GVHD) after first-line treatment with AZA or BSC is 25.0% (18.9–36.1%) and 30.0% (22.5–40.6%), respectively. Eighty-one (75.7%) patients turned MRD-negative after IFN-α treatment, including 42 (39.3%), 6 (5.6%), 7 (6.5%), and 26 (24.3%) who turned MRD-negative 1, 2, 3, and >3 months after MRD-directed IFN-α treatment, respectively. Twelve patients who relapsed after IFN-α treatment, and 4 patients died of non-relapse mortality (NRM). The 2-year cumulative incidence of relapse and NRM after IFN-α treatment was 11.5% and 4.3%, respectively. The 2-year probabilities of event-free survival and disease-free survival after IFN-α treatment were 66.5% and 82.4%, respectively. Persistent MRD after IFN-α treatment was significantly associated with higher relapse risk and poorer survival.

Summary/Conclusions: These data confirmed that MRD-directed IFN-α treatment is effective for patients who were MRD-positive after allo-HSCT.

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IMPACT OF AZACITIDINE PRETREATMENT ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROME

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous myeloid stem cell disorder with ineffective hematopoiesis, dysplastic cell morphology, and a propensity for progression to acute myeloid leukemia. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative therapy for MDS. In recent years, azacitidine (AZA) has been increasingly used as pre-transplant induction therapy in high-risk MDS patients. However, the benefits of pretransplant therapy in these patients are unclear, and the optimal therapy regimen remains unknown.

Aims: We conducted a retrospective analysis to elucidate the clinical impact of treatment with AZA on outcomes after allo-HSCT in high-risk MDS patients.

Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. We selected patients with high-risk MDS at diagnosis (IPSS intermediate 2 or high), aged 16 years or older, who underwent their first transplantation between January 2009 and December 2014 and received AZA or best supportive care (BSC) before allo-HSCT. Patients who received conventional chemotherapy or immunosuppressive therapy prior to allo-HSCT were excluded. We compared overall survival (OS), relapse, non-relapse mortality (NRM), and hematopoietic recovery after allo-HSCT between AZA and BSC patients. OS was estimated by the Kaplan–Meier method, and a log-rank test was used for comparisons. Relapse and NRM were considered competing risk events and were compared using Gray’s test. The cumulative neutrophil and platelet recoveries were also compared by Gray’s test, considering death without these events as a competing risk. In a 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 match, and prior therapy 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 failure as an indication for allo-HSCT was more frequent in AZA patients (P=0.02) than those in BSC patients. No differences were observed in other factors. The 2-year OS rate (46.7% and 50.8%, P=0.66), relapse (31.5% and 28.6%, P=0.59), NRM (26.5% and 26.1%, P=0.99), 30-day neutrophil engraftment (88.2% and 83.6%, P=0.18), and 60-day platelet engraftment (72.0% and 69.4%, P=0.36) were not significantly different between the AZA and BSC groups. In multivariate analysis, AZA and BSC showed comparable OS (HR, 1.16; P=0.31), relapse (HR, 1.13; P=0.50), NRM (HR, 0.92; P=0.64), neutrophil engraftment (HR, 1.01; P=0.89), and platelet engraftment (HR, 1.07; P=0.59).

Conclusions: AZA and BSC provided similar outcomes of allo-HSCT in high-risk MDS patients. Further analysis is needed to clarify the role of pretransplant therapy in high-risk MDS and to identify the subset of patients who may benefit from pretransplant AZA.
LOW-DOSE DECITABINE IMPROVES PLATELET RECOVERY IN PATIENTS WITH ISOLATED THROMBOCYTOPENIA AFTER HSCT

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Background: Isolated thrombocytopenia is a common complication of hematopoietic stem-cell transplantation (HSCT), which was defined as consistent low platelet counts with recovery of the other two cell lines after transplantation. This status leads to an increased risk of life-threatening hemorrhage, frequent requirements of platelet transfusion and extended hospital stays, representing a challenging clinical problem. Previous studies have demonstrated that decitabine, a hypomethylating agent, may increase platelet counts by promoting megakaryocyte maturation and platelet release in mouse model.

Aims: In order to investigate the role of decitabine in patients after HSCT suffering from isolated thrombocytopenia, we conduct a clinical trial to validate this effect in post-HSCT setting.

Methods: We performed a prospective open-label study to evaluate the treatment of low-dose decitabine in patients with hematological malignancies who received allologeneic HSCT and suffered from isolated thrombocytopenia. The inclusion criteria were: (1) Platelet count <30 × 10^9/L persistently at day 60 post-HSCT or later; (2) Recovered neutrophil and hemoglobin; (3) Full donor chimerism; and (4) No response to conventional treatments for a duration of at least 4 weeks. Patients with malignancy relapse, active infections, uncontrolled graft-versus-host disease, severe organ damage or transplant-related thrombosis were excluded. From July 2013 to July 2016, 38 patients were randomly assigned into either the control group to receive conventional treatment only, or the test group to receive additional decitabine (15mg/m2, intravenously daily for 4 to 6 weeks).

Results: Major response was observed in 16 out of 19 patients (84.2%) in decitabine group, with a median time of 22 days to achieve platelet transfusion-independence. Two patients (10.5%) showed a minor response and 1 patient (5.3%) failed. In contrast, 3 out of 19 patients in the control group (15.8%) showed a major response, 2 patients (10.5%) showed a minor response, 14 patients (73.7%) did not show any improvement, of which 1 patient died of severe hemorrhage in week 5. For bone marrow morphological analysis, all 38 patients showed low levels of megakaryocytes at week 0. However, the megakaryocyte counts in decitabine group were significantly increased at week 4, while no significant difference was recorded in control group. After decitabine treatment, we did not observe a change in anti-platelet antibodies levels and T cell subsets ratios. However, reactive oxygen species (ROS) and megakaryocyte counts increased in the test group. No considerable myelosuppression, febrile neutropenia, and nonhematologic toxicities associated with the treatment were observed.

Summary/Conclusions: Our data showed an encouraging efficacy of decitabine in patients after HSCT suffering from isolated thrombocytopenia. However, reactive oxygen species (ROS) and 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.

Phthalassemia

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QUANTITATIVE PROTEOMICS OF PLASMA EXTRACELLULAR VESICLES TO IDENTIFY NOVEL BIOMARKERS OF CLINICAL SEVERITY FOR HBE/B-THALASSEMIC PATIENTS

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Background: Hemoglobin (Hb) E/B-thalassemia has a wide spectrum of clinical manifestations that cannot be explained purely by its genetic background. Extra- cellular vesicles (EV) are one factor that may indicate and/or contribute to disease severity because there is an observed increase in EV release due to the enhanced oxidative stress in thalassemic erythrocytes.

Aims: This study aims to explore the differences in protein composition and abundance between circulating EV from HbE/B-thalassemic patients and normal individuals.

Methods: 15 HbE/B-thalassemia patients and 15 matched-controls from Thailand were fully consented and recruited for this study. Pooled EVs isolated from five thalassemic samples were compared to pooled EVs from five matched controls using a Duplex-Tandem Mass Tag (TMT) mass spectrometry (TMT-MS) analysis. This experiment was repeated three times in total, using different patient and control samples to identify consistent alterations of protein expression in EVs. Finally, protein differences were also confirmed using Western blotting.

Results: The total proteins identified across the three experimental TMT-MS datasets ranged from 1.764 to 2.534 proteins. When restricted to proteins that contained more than one unique peptide, the range of proteins was reduced to 685 to 1,127 proteins. Many proteins were previously reported EV constituents. 19 proteins were consistently increased in patient samples compared to controls across all data sets. The majority of these proteins were chaperone proteins and antioxidant enzymes. Alpha Hemoglobin Stabilizing Protein (AHSP) had the highest increase of between 31 to 47-fold. Other proteins that exhibited increased abundance in thalassemic circulating EV included catalase, superoxide dismutase, T-complex proteins, heat shock protein 70 and ferritin light chain. Importantly, the heme scavenger and plasma proteins – haptoglobin and hemopexin were observed to be consistently decreased in patients’ EV across all data sets. Immunoblotting results corroborated the TMT-MS findings.

Summary/Conclusions: We have successfully identified consistent alterations in protein expression levels between EV generated by HbE/B-thalassemic patients and normal individuals. These findings may potentially lead to the development of a prognostic marker, and therefore may improve the therapeutic outcome for the patients suffering from thalassemia.
and in the percentage of circulating erythroblasts; (iv) the increase in β Thal red cell survival. RO4917838 induced a significant reduction in extramedullary erythropoiesis as well as in the amount of insoluble alpha chain aggregates in circulating red cells. It is of note that in β-Thal sorted erythroblasts we found a reduction in HRI and in phospho-eIF2α, 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 β-thalassemia.

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MAY MUTATIONS IN THE KLF1 GENE HAVE WORSENING EFFECTS ON THE BETA THALASSEMA 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 β-globin gene cluster and the fetal-to-adult globin gene switching (Wayne JS et al Int. J. Lab. Hem. 2015; 37: 78-84). It has been reported that mutations leading to KLF1 haploinsufficiency cause a wide spectrum of diseases ranging from the condition of a neutral polymorphism. Furthermore, unexpectedly, the novel mutations in the KLF1 gene associated with atypical β-thalassemia phenotypes were linked to increased fetal hemoglobin (HbF) levels with ameliorative effects on the severity of β-thalassemia (Liu D. et al. Blood 2014; 124: 803-811; Perkins A. et al. Blood 136: 1178-1189). It has been reported that mutations leading to KLF1 haploinsufficiency cause a wide spectrum of diseases ranging from the condition of a neutral polymorphism. Furthermore, unexpectedly, the novel mutations in the KLF1 gene associated with atypical β-thalassemia phenotypes were linked to increased fetal hemoglobin (HbF) levels with ameliorative effects on the severity of β-thalassemia (Liu D. et al. Blood 2014; 124: 803-811; Perkins A. et al. Blood 2014; 127: 1856-1862). Aims: This study was aimed at providing a functional characterization of known and novel mutations in the KLF1 gene associated with atypical beta-thalassemia phenotypes.

**Methods:** Hematological parameters were measured using an automated hematology analyzer (Beckman Coulter) and high performance liquid chromatography (Variant II, Bio-Rad Laboratories). Screening of KLF1 mutations was performed by Sanger sequencing on an Applied Biosystems 3730 DNA analyzer. Functional studies were performed by gene reporter assays and expression vectors for KLF1 mutants in the human K562 erythroleukemia cell line. This study was performed on 19 adult subjects, including 11 beta-thalassemia heterozygotes with an unexpected phenotype of intermediate thalassemia and 8 subjects with nonβ erythocyte indices and borderline HbA2, HbF and/or HBF levels without mutations in alpha- and beta-globin gene clusters.

**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 nucleotide variations (c.-148 G>A, c.182 C>T and c.183 C>T) in the coding region and 2 nucleotide variations (c.124 A>G and c.135 T>C) in the 3′UTR. These mutations were found to be associated with increased fetal- and/or beta-globin gene expression. In the 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.

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SECONDARY SOLID TUMORS FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION FOR THALASSEMA 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 adult patients 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 the 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 received allogenic bone marrow transplantation (median age at time of HCT including 21 transplanted patient population. Two patients developed an hepatocellular 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 an age- and sex matched nontransplant TM patients or with stem cell donor. Notably, among the transplanted patients we did not 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 (NBS-Hbs) is crucial for early detecting patients with serious hemoglobinopathies (Hb variants) e.g. sickle cell anemia (Hb SS). NBS-Hbs has been incorporated into a routine neonatal service in several developed countries. However its role on early detection other forms of globin disorders remains unclear. Moreover, NBS-Hbs can detect several types of thalassemia and Hb variants carriers. This application could be useful for the national prevention and control programs since thalassemia syndromes in many developing countries including Thailand where these conditions are highly prevalent especially β-thal major. Hb E/β-thal and Hb Bart’s hydrops fetalis (caused by α°-thalassemia). Recently a new capillary electrophoresis (CE) has been developed specifically for NBS-Hbs. However there is a limited data on validation of this technology on detecting several types of thalassemia and Hb variants found in Southeast Asia.

Aims: To evaluate and validate a new CE system to screen globin disorders in newborn to initiate the national NBS-Hbs for Thailand

Methods: After informed consent, 1,213 blood samples of 2-day old newborns were collected by heel prick puncture into 5-dried blood spots. After elution, dried blood samples were analyzed by Capillaries 2 NEONAT FAST® (SEBIA, Evry, France). All samples were also extracted for DNA and genotyped by our extensive PCR based panel to detect >96% of abnormal globin alleles found in Thailand using α-thal GAP-PCR, α-thal ARMS-PCR, β-thal ARMS-PCR, and PCR-RFLP for Hb E. We compared CE data with each globin genotypes and use a ROC curve to set up new diagnostic criteria using% Hbs from CE for future cases.

Results: Identification of Hb Bart’s provided 100% 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%, and ≥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°-thalassemia (Hb Bart’s) ≥20.10% (detectable level). A cut-off level to distinguish Hb EE from Hb E trait was suggested at ≥3.35% vs ≤1.67%.

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). Six patients with normal α globin genotypes (n=148). We recommend Hb A level ≤10.35%; a cut-off to primarily consider for β-thalassemi carrier followed 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 NBS into a routine service in order to early detect Hb H disease, Hb E/β-thalassemia and the majority of common thalassemia carriers. This NBS-Hbs approach can reinforce and leverage our current program on prevention and control for severe thalassemia syndromes in our region. Moreover, due to population migration from The East to the West, our new diagnostic guideline by CE could be useful and applicable for existing NBS programs currently available in several European countries.

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TRANSIENT ELASTOGRAPHY IN NON TRANSFUSION DEPENDENT THALASSEMA: A SUCCESSFUL TOOL TO ASSESS AND MONITORING LIVER FIBROSIS

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Background: Non Transfusion Dependent Thalassemia (NTDT) patients are at risk for several complications due to chronic anemia, hypoxia and iron overload. Over the recent years hepatic complications are more frequently observed in these patients probably due to the aging and poor care: monitoring liver fibrosis is becoming part of the follow up. Liver stiffness measurement (LSM) by transient elastography (TE), a widely-used non-invasive tool, in our centre has been included in the regular follow-up of patients with NTDT

Aims: To evaluate by TE liver fibrosis in NTDT patients, its correlation with biochemical, hematological and clinical parameters as baseline and after 5 years

Methods: Hepatic fibrosis and siderosis were evaluated in 101 NTDT patients using respectively TE, and liver iron concentration (LIC) derived from T2 Magnetic Resonance Imaging (MRI) at baseline and, in a subset of patients, after 5 years. The following TE thresholds were taken into account: <5.0 kPa no fibrosis (F0), 5.1-7.9 kPa mild fibrosis (F1), >7.9 kPa moderate fibrosis (F2), >10.3 advanced fibrosis (F3), >11.9 kPa cirrhosis (F4). Biochemical and hematology blood test were collected too. Patients were also tested for HCV antibodies and HCV RNA. Data were analyzed retrospectively.

Results: Patient’s mean age was 46±11 years, 37/101 (36.6%) were splenectomized, 51/101 (50.5%) had never been transfused, 46/101 (45.5%) were occasionally transfused and 4/101 (3.9%) had been regularly transfused for 10±5yrs. At baseline (T0), the overall mean LSM was 5.9±2.6 kPa, median 3.65 (IQR 2.85-7.45), LIC >593 ng/ml was found in 48/60 (79.9%) patients. The mean LIC at baseline was 622±201 ng/ml, mean LIC at 5±1 years (T1) were available. LSM remained stable in 35/60 (58.3%): 24/60 (40%) patients had no fibrosis (F0) at T1, 7/60 (11.7%) had F1, and 4 patients (6.7%) were on ICT. At baseline 37/101 (36.6%) patients were on iron chelation (ICT) (29 deferoxamine, 7 deferasirox, 1 deferiprone). At T0 patients with fibrosis (any grade) didn’t show differences compared with patients without fibrosis (F0) regarding age, splenectomy, transfusions, ICT and all biochemical parameters. LIC >593 ng/ml at T0 was significantly associated with F1 fibrosis at both evaluation (OR=2.71, 95% CI 1.0 to 7.30). LIC >393 ng/ml at T0 was significantly associated with F2 fibrosis at both evaluation (OR=3.24, 95% CI 1.0 to 10.20).

Among these patients 13/35 (37.1%) were on ICT. A reduction in LSM was found in 21/60 (35%) patients (T0=7.09±1.63 kPa, T1=5.07±1.61 kPa, p<0.001), with a reduction trend in LIC (T0=6.79±4.09 mg/dl, T1=5.18±3.04 mg/dl; p=0.09 ns) and a statistical significant reduction in ferritin levels (T0=709±568 ng/ml, T1=436±280 ng/ml, p<0.005). 21 (42.6%) were on ICT. Furthermore, patients who were on iron chelation had significantly higher LIC levels than patients who were not on chelation. There were no significant differences in the mean LIC at baseline and T1 (T0=668±596 ng/ml, T1=567±473 ng/ml, p=0.108).

Conclusions: Liver stiffness measurement (LSM) and transient elastography (TE) are non-invasive methods that can be very helpful in the follow-up of NTDT patients, especially when they are on iron chelation. These non-invasive methods can contribute to the early detection of progressive liver fibrosis and could be used to monitor the treatment effect on the reduction of LM in NTDT patients. In addition, the results obtained in this study highlighted the need for more research on the early detection and management of liver fibrosis in NTDT patients.
with malignancies were identified (incidence: 4.6%). The mean age of the diagnosis of the malignancy was 41.8 years (36.6 years for thyroid gland cancer, 45.8 years for liver, 38 years for hematologic malignancies and 46 for renal cancer). 24 patients were transfusion dependent (TD) (7% of the patients) and 3 non transfusion dependent (1.18%). Liver cancer had the highest incidence 29.6%, following by thyroid gland cancer 25.9%, hematologic malignancies 11.1% and renal cancer 14.8%. HCV infection was found in 56.7% of the patients and a statistical significant relationship between HCV infection and cancer (p<0.001) was detected. No correlation between liver failure and cancer was detected. In the TD group, the age specific ratio of cancer increased with age with the patients >50 years having the highest ratio of 42.3, compared to 36.6 in the TD <50 years and 41.45 years age group, respectively. In regards to chelation therapy, at the time of diagnosis 40.9% of the patients were receiving deferasirox (DFX), 22.7% deferiprone (DFP), 22.7% deferoxamine (DFO), 9.1% no chelation therapy and 4.5% DFO/DFP. No statistical significant difference was observed between the different chelation therapies. Throughout the years, according to the availability of the chelating agents, we analyzed separately, the patients that developed malignancies in the period after 2010 when longitudinal exposure to all three chelators can be assumed. Even though the results showed a difference (p<0.027) between the different groups with 47.1% of those patients receiving DFX at the time of the diagnosis compared to 27.1% receiving DFP and 11.8% receiving DFO, this distribution reflects the overall distribution of chelator usage during that period. Apart from the incidence, there was no statistical significant difference between TD and NTD patients with cancer regarding the gender, age and year of diagnosis. The overall cancer mortality rate was 48%, but varied significantly with the type of cancer with liver cancer and hematological malignancies having a mortality of 66%. Overall only 2% of the deaths occurring in our group of patients were attributed to cancer.

Summary/Conclusions: This retrospective study has confirmed the increased incidence of malignancies in thalassemia patients in Greece, which is, at least, partially related to the aging of this population. Based on these observations, adaptation of monitoring guidelines is essential for optimal management of thalassemic patients. Periodic screening for malignancies, especially hepatic, thyroid and hematologic, will allow early detection and timely, and thus, more efficacious treatment of the neoplasia.

P398
SAFETY AND EFFICACY OF EARLY START WITH SUBOPTIMAL DOSE OF DEFERIPRONE IN MINIMALLY TRANSFUSED INFANTS WITH TRANSFUSION DEPENDENT THALASSEMA: 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 practice 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 deferoxamine 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), sixty-four children recently diagnosed with thalassemia major who had begun receiving blood transfusions in first year of life to keep pre-transfusion Hb above 10 gm/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 8 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 µg/L. Mean ± SD time of follow up was 10.44±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 therapy. 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.

Figure 1.

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.

P399
LONGITUDINAL PROSPECTIVE MRI STUDY IN PEDIATRIC PATIENTS WITH THALASSEMA MAJOR
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Background: No studies are available in literature evaluating, on repeated measurements, 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

Table 1.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>8 months</th>
<th>10 months</th>
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<tr>
<td>SF (µg/L)</td>
<td>ES-07</td>
<td>43.5±8.2</td>
<td>42.5±4.5</td>
<td>41.8±2.8</td>
<td>41.3±2.8</td>
<td>41.4±2.8</td>
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<tr>
<td>DC-07</td>
<td>39.0±2.8</td>
<td>31.8±2.8</td>
<td>30.4±2.8</td>
<td>30.0±2.8</td>
<td>30.1±2.8</td>
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<td>31.8±2.8</td>
<td>30.4±2.8</td>
<td>30.0±2.8</td>
<td>30.1±2.8</td>
<td>30.1±2.8</td>
</tr>
<tr>
<td>% patients with SF ≥1000 µg/L</td>
<td>ES-07</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>DC-07</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>ES-07</td>
<td>64.0±6.8</td>
<td>64.2±6.8</td>
<td>64.2±6.8</td>
<td>64.2±6.8</td>
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<tr>
<td>DC-07</td>
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<td>64.2±6.8</td>
<td>64.2±6.8</td>
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<tr>
<td>LPI (µM)</td>
<td>ES-07</td>
<td>0.28±0.02</td>
<td>0.13±0.02</td>
<td>0.24±0.02</td>
<td>0.26±0.02</td>
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<td>DC-07</td>
<td>0.28±0.02</td>
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<tr>
<td>% patients with LPI ≥0.6 µM</td>
<td>ES-07</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>DC-07</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>5%</td>
<td>10%</td>
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</table>

Table 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 the 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-TALASSEMA 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 deferiprone (basal 30%>f.u.32%), deferoxamine (basal 45%>f.u.52%), daily alternating deferasirox+deferoxamine (basal 3%>f.u.6%), deferoxamine+ deferoxamine (basal 9%>f.u.6%) and heart (Rp=0.68, p<0.001) and pancreas (Rp=0.75, p<0.001), in accordance with literature. Moreover, the ROC analysis confirms the value of 100 Hz for the pancreatic-R2* as the predictor of a cardiac R2*>50Hz, we calculated the numbers of false/true positive/negative according to the rule above. At the baseline we can observe that the number of false positive is the 14/27 (52%). The percentage increases to 91% (21/23) after f.u. the pancreas-R2*>100Hz in 23 patients but only 2 has iron overload in the heart, the total number of patients with pancreatic-R2*>100Hz is quite the same before and after f.u. (27 compared to 23). We found no correlation between the false positive predicted and particular conditions such as impaired glucose tolerance, diabetes or adipose involution (Table 1).

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 regiment (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 infection.

**Aims:** The aim of this study was to develop an effective HTLV-1 hyperimmune globulin (HTLV-IG) isolated from HTLV-1 positive carriers screened at the Japanese Red Cross.

**Methods:** We developed two in vitro and in vivo screening methods to evaluate and characterize the anti- viral effect of HTLV-1 positive plasma and HTLV-IG.

**Results:** HTLV-1 positive plasma 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 Il2rgtm1Sug/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-1 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 and in vivo infection assays. We next assess the viral safety of HTLV-1 during the HTLV-IG manufacturing process. High log reduction values of HTLV-1 can be seen during the Cohn fractionation process. Virus safety was assessed with PCR based assay and in vitro and vivo infection assay.

**Summary/Conclusions:** These data suggest HTLV-IG is effective and safe for the prevention of HTLV-1 infection.

P402

THE COMANITATION OF TUMOR CELLS IN THE APERATURESIS MATERIAL DOES NOT PREDICT THE RESPONSE OF MULTIPLE MYELOMA PATIENTS TO AUTOLOGOUS TRANSPLANTATION

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1Hematology and Clinical Oncology, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CB15/00055-CIBERER, Murcia, Spain

**Background:** The use of high dose of chemotherapy followed by autologous stem cell transplantation (ASCT) has improved the prognosis of patients with multiple myeloma (MM) and plasma cell dyscrasia. However, there is controversy over the effect of infusion of atypical plasma cells (PC) on the apheresis product.

**Aims:** To analyze whether in MM malignant plasma cell reinfusion could negatively affect responses to ASCT.

**Results:** At day +100 following TPE, which almost always corrected by the next day.

**Summary/Conclusions:** Infusion of PC with atypical phenotype does not appear to affect the response at day+100 following ASCT, in patients with MM or plasma cell dyscrasia. Conversely, the quality of response to induction therapy was significantly associated to 100-day outcome after transplantation. These data support that in vivo persistent residual cells, but not those being infused with the graft, are the main source of relapse in MM.
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 TPE courses 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 with involved service provision, and strengthening of the role of apheresis nurse as lead.

Results: Of 141 participants who took part in the survey, 31% (43) had been qualified for less than two years and 47% (65) were consultants. Specialties included Surgery, Anesthesiology, 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 57g/L for transfusion in patients without acute coronary syndrome with an ISSC score of 2. For patients with a threshold of 85g/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 the threshold of 47g/L (66) routinely measuring this prior to transfusion, and only 31% (44) aware that a ferritin result over 30 days old should be rechecked. This highlighted potentially inadequate identification of iron deficiency anemia. In addition only 40% (57) were aware of the existence of a hospital anaemia clinic for referral. When reflecting on consent methods, 96% (135) of participants expressed the indication for transfusion, and 90% (127) gave an opportunity to ask any questions and ensured the patient was content to proceed. Provision of written information was poor (26%, 37) and only 55% (78) recorded the discussion in patients’ notes. Exploring barriers to consent, 24% (32) expressed difficulty in obtaining a patient information leaflet, and issues relating to lack of time and 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 highly 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 with one of the hospital transfusion teams which will disseminate the survey to all hospital staff, and carrying out structured case based discussion sessions with junior doctors to enhance knowledge and confidence.
Front-line combinations in multiple myeloma and amyloidosis

S407
QUADRUPLET 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 intensifying therapy for those with a suboptimal response. 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 d1-2,9,15-16 (20mg/m² #1d-1-2), cyclophosphamide (cyclo) 500mg PO d1,8,15, lenalidomide (len) 25mg PO d1-21, dexamethasone (dex) 40mg PO d1-4,8-9,15-16). Patients were randomised to this up-front quadruplet or the sequential strategy of CRD (cyclo 500mg PO d1,8, len 25mg PO d1-21 PO daily, dex 400mg PO d1-4,12-15) or CTD (cyclo 500mg PO d1,8,15 thalidomide 100-200mg PO daily, dex 40mg PO d1-4,12-15) given to maximum response. Patients with VGPR/CR proceeded straight to ASCT, those with PR/MR were randomised to sequential CVD (cyclo 500mg d1,8,15, bortezomib 1.3mg/m² IV/S SC d1,4,8,11, dex 20mg PO d1,2,4,5,8,9,11,12) or nothing and those with SD/PD all received sequential CVD. At day 100 post ASCT there was a maintenance randomisation between lenalidomide and observation. The trial has now closed to recruitment and all patients have completed induction therapy. This analysis compares responses and toxicity of the different regimens.

Table 1.

<table>
<thead>
<tr>
<th>Table</th>
<th>Treatment exposure and safety data.</th>
<th>All patients who did not receive SCT (n=42)</th>
<th>AE with onset during induction (cycles 1-12)</th>
<th>AE with onset during maintenance (cycles 13-24)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>AE with onset during maintenance (cycles 13-24)</td>
</tr>
</tbody>
</table>

Results: 2568 TE patients underwent induction randomisation (CTD 1021, CRD 1021, KCRD 526). Patients were comparable with respect to age (median 59 years), sex and other key laboratory parameters. Patients were mandated to complete 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 ≥3 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.
Methods: In this phase 1/2 study (NCT01217957), patients with NDMM received weekly oral ixazomib (1.83-3.5mg/m²; days 1, 8, and 15) plus lenalidomide (25mg, days 1-21) and dexamethasone (40mg, days 1, 8, 15, and 22) for up to 12-28 week induction cycles, followed by maintenance therapy with weekly single-agent ixazomib, at the last tolerated dose given during induction, until disease progression or toxicity.

Results: Of the 42 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 (COR; ≥ partial response [PR]) 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 years after SCT. Median PFS and OS for patients who received maintenance therapy was 24 months. The occurrence of the most common treatment-related grade ≥3 AEs (neuropathy, 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. Rd followed by single-agent ixazomib maintenance also showed an acceptable safety profile, with less toxicity reported during the maintenance (single-agent ixazomib) vs induction (Rd) periods, with no evidence of cumulative toxicities.
Table 1.

<table>
<thead>
<tr>
<th>Grade 3-4 AEs/SAEs</th>
<th>KCd</th>
<th>KRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological</td>
<td>13%</td>
<td>9%</td>
</tr>
<tr>
<td>Cardiac</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>Thromboembolism</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0%</td>
<td>3%</td>
</tr>
<tr>
<td>AST/ALT/GGT increase</td>
<td>6%</td>
<td>9%</td>
</tr>
<tr>
<td>Dermatological</td>
<td>6%</td>
<td>9%</td>
</tr>
<tr>
<td>Infections</td>
<td>6%</td>
<td>9%</td>
</tr>
<tr>
<td>Acute Kidney Injury</td>
<td>p value &lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: Safety profile was acceptable; more patients required plerixafor in the KRd arm. Rate of VGPR was higher with KRd. Updated data on a higher number of patients will be presented at the meeting. The trial is registered at Clinicaltrials.gov: NCT02203643

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 1-2 and ejection fraction >45% were included. Major exclusion criteria were symptomatic orthostatic hypotension, NT proBNP level >5000 pg/ml, Troponin T > 0.06 ug/l, Bilirubin >2x ULN, 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/m2 twice a week in a 21-day cycle, D 20mg orally on each B day and the following day. HDM dosage was 200mg/m2. Hematological responses were defined according to consensus criteria with the addition of very good partial response (VGPR), defined as the difference between involved and uninvolved FLC<40mg/L. Cardiac, renal and liver response and progression criteria were defined according to consensus criteria with addition of NT proBNP.

Results: Median age was 59 years (range 26-70) and 60% were male. NYHA stage 1 was 56% and 2 in 42% of patients. Mayo cardiac risk score was I (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.
Hodgkin and indolent lymphoma - Clinical

S412

NIVOLUMAB FOR RELAPSED/REFRACTORY CLASSICAL HODGKIN LYMPHOMA AFTER AUTOLOGOUS TRANSPLANT: FULL RESULTS AFTER EXTENDED FOLLOW-UP OF THE MULTICOHORT MULTICENTER PHASE 2 CHECKMATE 205 TRIAL


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Background: Nivolumab, a fully human IgG4 monoclonal antibody targeting programmed death-1, is an immune checkpoint inhibitor that augments T-cell activation and antitumor responses. Nivolumab is indicated for pts with relapsed/refractory (RR) classical Hodgkin lymphoma (cHL) following autologous stem cell transplantation (ASCT) and brentuximab vedotin (BV) treatment. The multicohort phase 2 CheckMate 205 trial (NCT02181738) enrolled pts with cHL for relapse after ASCT in CheckMate 205.

Methods: This was a single-arm, multicenter trial enrolling pts (age ≥18 y) with RR cHL after ASCT into 1 of 3 independent cohorts (Cohort A: BV-naive; 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 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 evaluated in pts with progressive disease after failure of ASCT due to their limited treatment options.

Aims: To report extended follow-up data for all pts with RR cHL after failure of ASCT in CheckMate 205.

Results: In total, 243 pts were treated: 63 in Cohort A (BV-naive), 80 in Cohort B (BV after ASCT), and 100 in Cohort C (BV before [n=53], after [n=58], or both after [n=32]). Median (range) age was 39 (16-72) y and 77% of pts had advanced (stage III+) disease at study entry. BV-naive 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-naive 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 AEs were fatigue (23%), diarrhea (15%), infusion reactions (IRs; 14%), and rash (12%); grade 3-4 drug-related AEs in ≥3% of pts were lipase increases (5%), alanine aminotransferase increases (3%), and neutropenia (3%). The most common drug-related serious AEs were IRs (2%) and pneumonitis (1%). To facilitate translation to practice, efficacy results by sequencing of prior BV treatment will also be presented.

Summary/Conclusion: With extended follow-up, high and durable rates of CR and PR to nivolumab therapy were observed in pts with RR cHL after ASCT, irrespective of BV treatment history.

Study funding: BMS; medical writing support: M. Thomas (Caudex), funded by BMS.

S413

EARLY CHEMOTHERAPY INTENSIFICATION WITH ESCALATED BEACOPP IN ADVANCED-STAGE HODGKIN LYMPHOMA WITH A POSITIVE INTERIM PET-CT AFTER 2 PET-CT CYCLES: LONG-TERM RESULTS OF THE GITL/FIL HD 6607 TRIAL


Background: Interim 2-[18F]fluoro-2-deoxy-D-glucose Positron Emission Tomography (FDG-PET) performed after 2 chemotherapy cycles (PET2) is the most powerful predictor of treatment outcome in ABVD-treated, advanced-stage classical Hodgkin Lymphoma (cHL). Preliminary reports of early treatment intensification adopting PET2 result could increase the efficacy of standard ABVD.

Aims: To confirm in a prospective setting the favorable prognosis of advanced stage PET2 negative patients treated with ABVD, as well as the safety and efficacy of escalated BEACOPP given to PET2 positive patients.

Methods: We conducted a prospective clinical trial (HD9067 ClinicalTrial.gov identifier 00795613), in which advanced-stage (IIB-IVB) cHL patients were treated with 2 ABVD courses, and PET2 performed afterwards. The latter was blindly and independently reviewed by a panel of nuclear medicine experts, using the Deauville 5-point scale (5-PS). PET2+ patients (5-PS 4-5) were randomized to either BEACOPP escalated (Be+BB) or BEACOPP baseline (Be) plus BEACOPP baseline (Be+BB) or Be+Eb (4+4) and Rituximab (R). PET2- (5-PS 1-3) patients continued ABVD treatment with 4 more cycles and, upon CR achievement, randomized to either consolidation radiotherapy (Rtx) on the sites of initial large nodal mass (LNM: diameter >5cm) or no further treatment (NFT).

Results: From June 2014 till June 2017, 145 cHL patients were consecutively enrolled in 24 Italian and 1 Israeli centers. The median age was 31 years (14-60); 35% had stage IIB, 32% stage III and 32% stage IV. The International Prognostic Score (IPS) was 0-1 in 36.6%, 2-3 in 51%, >3 in 12.5%.
Overall, 150 (19.2%) proved PET2+ (97 score 4, 53 score 5) and 630 (80.5%) PET2-. 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. Out of 296 with LNM, 148 were randomized to RxT and 148 to NFT. Among 627 patients, 574 (91.5%) achieved CR, 50 (8.0%) had a treatment failure and 3 (0.4%) 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 83% (95% CI 80%>86%) 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 89% (95% CI 82%>93%) 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%>76%), respectively (p=0.9731). Consolidation RxT in PET2- patients in CR after 6 ABVD and LNM did not translate in to a significant benefit, with a 4-y PFS of 96% (95% CI 91%>98%) for RxT and 93% (95% CI 87%>96%) for NFT (p=0.288).

**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 RxT in PET2- 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 RxT in PET2- cHL; 4) the addition of Rituximab does not include LDH, which is a well identified prognostic factor both in lymphomas and multiple myeloma.

**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 21th of June 2013. The sample consisted of 51 male (74%) and 18 female (26%) patients. At time of 3rd relapse the age of the patients ranged from 20 to 79 years (mean 39.2 years, standard deviation (SD) 14.0 years) and the majority of patients presented with stage III or IV disease (67%). Time from end of 3rd-line treatment to 3rd relapse was ≤3 months (i.e. GHSG definition of refractory disease) in 15 cases (22%), ≤12 months (early relapse) in 19 cases (28%) and >12 months (late relapse) in 35 cases (51%). All 69 patients were pretreated with chemotherapy, 35 (50.7%) with BEACOPP, 30 (43.5%) with ABVD and no BEACOPP, and 32 (46.6%) with another type of chemotherapy. The number of prior chemotherapies ranged from one to three (median 3). Pretreatment with radiotherapy was observed in 57 (82.6%) patients, with salvage chemotherapy aimed to induce a remission prior to a stem-cell transplantation (SCT) in 58 (84.1%), and with high dose chemotherapy followed by autologous SCT in 50 (72.5%) patients. Four patients (5.8%) had received allogeneic SCT as 3rd-line treatment. None of the patients had received brentuximab vedotin or anti-PD1 antibodies before 3rd relapse. With a median observation time of 63.3 months for OS after 3rd relapse, 45 patients (65.2%) had died and 60 (87.0%) had another PFS event. Twelve months after the 3rd relapse OS was 73.2% (95%-CI 62.6% to 83.8%) and PFS 50.8% (95%-CI 38.9% to 62.8%, Table 1).

**Table 1.**

| Figure 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 MACROGOBLU-LEINMA**

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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 monoclonal serum immunoglobulin M (IgM) immunoglobulin in the serum. It 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, lgM levels and b2 microglobulin) and stratifies WM patients into 3 broad risk groups. IPSSWM does not take into account non-IgM eust-length 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.**

| Programmed Free Survival (PFS) Overall Survival (OS) |
|---|---|---|---|---|---|---|
| | % | 95% confidence interval | % | 95% confidence interval |
| 6 months | 16 | 76.3 | 61.5% | 85.4% | 7 | 89.6 | 82.4% | 96.9% |
| 12 months | 53 | 10.8 | 13.9% | 62.6% | 18 | 73.2 | 62.0% | 83.8% |
| 18 months | 47 | 29.3 | 18.5% | 69.2% | 25 | 64.5 | 54.3% | 77.0% |

**Summary/Conclusions:** Patients with a 3rd relapse or progression of WM 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, with minimal toxicity and is recommended as the treatment of choice for this disease related. R therapy was well tolerated. Only one pt could not complete treatment due to intolerance.

Table 1.

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.
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


Background: The JAK-STAT pathway is a critical controller of cellular proliferation, differentiation, survival and apoptosis in response to external stimuli. The 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 (Blew 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 5' direction, establishing a new reading frame. This directs ribosomes towards premature termination codons, resulting in destabilization of the -1 PRF signal-containing mRNA via nonsense-mediated mRNA decay (Figure 1). There is thus an inverse relationship between -1 PRF efficiency and mRNA stability.

Aims: To investigate whether the JAK2-V617F mutation, shown here to be located in the pseudoknot of a -1 PRF signal in the JAK2 mRNA, impacts disease progression through ablation of -1 PRF.

Methods: Computationally predicted -1 PRF signals were validated using dual luciferase reporters and proteomic analysis of 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 HEK293T expressing Ba/F3 cells, in vivo experiments were performed in BALB/c mice.

Results: We demonstrate in human cell lines that the JAK2-V617F mutation structurally disrupts the -1 PRF signal in the JAK2 mRNA, leading to ~2-fold lower rates of -1 PRF and increased abundance of the JAK2 mRNA and protein. The transforming potential of a series of mutants designed to manipulate -1 PRF independent of V617F was assayed in a Ba/F3 cell model. Silent protein coding changes in the pseudoknot of the -1 PRF signal at position V617 (V617m) or the slippery site (SSm), both of which reduced frameshifting, increased JAK2 expression and led to transforming activity, albeit less than V617F. Importantly, the V617F+SSm combination conferred an additive effect on cellular transformation. Ba/F3 cells expressing these JAK2 variants were also introduced into mice. Whereas mice injected with wild type JAK2 remained healthy, both V617F and SSm induced similar leukemia phenotypes as V617F and V617F+SSm, with a ~2-fold longer disease latency of 8-10 weeks. Increased JAK2 mRNA abundance in JAK2-V617F homozygous patients as well as the presence of three additional -1 PRF signals in the JAK2 mRNA further suggest a prominent role for -1 PRF in controlling JAK2 production.

Conclusions: We demonstrate that the JAK2-V617F mutation diminishes -1 PRF on the JAK2 transcript, stabilizing the mRNA and increasing JAK2 expression, contributing to its transforming activity in vitro and disease onset in vivo. We suggest that -1 PRF normally provides a layer of control by limiting JAK2 translation. Defective -1 PRF synergizes with the transforming activity of the JAK2-V617F protein by causing its overexpression, explaining why this particular mutation causes such aggressive malignancies. In support of this, the combination of ruxolitinib and an 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).

Figure 1.
S420
JAK2 V617F HAEMATOPOIETIC CLONES WITH DIFFERENT EXPANSION KINETICS ARE DETECTABLE SEVERAL YEARS PRIOR TO MPN DIAGNOSIS
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Aims: To determine whether DAS is safe and effective in pediatric pts with CML-CP newly diagnosed or resistant/intolerant to IM enrolled in a phase 2, open-label, nonrandomized prospective clinical trial (CA180-226/NCT00777036).

Table 1.

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Clinical trials including treatment discontinuation in CML

S422

DASATINIB IN CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) FROM A PHASE 2 TRIAL


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Background: As safe and effective frontline treatment options for children and adolescents with CML are limited, and no approved therapies exist for patients (pts) resistant/intolerant to imatinib (IM), additional treatment options and alternative formulations are greatly needed for this younger population. Dasatinib (DAS) has proven efficacy in adults with newly diagnosed CML-CP, as well as those resistant/intolerant to IM (Cortes JCO 2016, Shah AJH 2016). Results of a phase 1 study confirmed its dosing and safety in pediatric pts (Zwaan JCO 2013); however, a larger prospective study is necessary to further support the use of DAS in pediatric pts with newly diagnosed or IM-resistant/intolerant CML-CP.

Aims: To determine whether DAS is safe and effective in pediatric pts with CML-CP newly diagnosed or resistant/intolerant to IM enrolled in a phase 2, open-label, nonrandomized prospective clinical trial (CA180-226/NCT00777036).

Summary/Conclusions: In this novel Ezh2-KO hJAK2V617F mouse model, Ezh2 loss collaborates to worsen thrombocytosis and rescue the HSC function defect in hJAK2V617F mice. We also observed a striking disruption of phenotypic and functional HSC heterogeneity in Ezh2-KO hJAK2V617F mice with an unexpected and selective loss of vwf-eGFP+ve HSCs together with subversion of vwf-eGFP–ve HSCs towards platelet-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.
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 comparable.

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 >30% was reached by 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-BP (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 pulmonary edema/hypertension or pulmonary arterial hypertension 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 to first- or second-line therapy 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.

S423
INITIAL REDUCTION OF THERAPY BEFORE COMPLETE WITHDRAWAL IMPROVES THE CHANCE OF SUCCESSFUL TREATMENT DISCONTINUATION IN CHRONIC MYELOID LEUKAEMIA (CML): YEAR 2 RESULTS IN THE BRITISH DESTINY STUDY
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Background: In CML, there is considerable current interest in whether some patients can safely discontinue tyrosine kinase inhibitor (TKI) therapy. However, all studies so far have examined patients in stable MR4 at entry, i.e., BCR-ABL1/ABL1 ratio ≤0.01%. Patients in stable major molecular response (MMR) but not MR4 (<0.1 but >0.01%) have not been formally studied, neither have the effects of stepwise TKI withdrawal.

Aims: The present British De-Escalation and Stopping Therapy with Imatinib, Nilotinib or sprycel (DESTINY) study examines treatment de-escalation as a prelude to complete cessation, in patients in not only stable MR4 but also those with MMR but not MR4.

Methods: Trial entry required first chronic phase of CML, TKI treatment for ≥3 years, and either the same TKI (imatinib, dasatinib or nilotinib) since diagnosis or only one switch for intolerance. All PCR tests (minimum of 3) in the 12 months before trial entry must have been ≤0.1% (i.e., MMR), each with ≤1,000 ABL1 copies/mL. All results meeting criteria were enrolled. all results ≤0.01% were enrolled. At entry, 14 patients had IM-resistant/intolerant, 14 nilotinib and 10 dasatinib, for a median duration of 6.8 years. We reported at ASH 2016 that after 12 months of half-dose therapy, molecular recurrence was lower in patients with stable MR4 at entry (3 of 125 patients; 2.4%) than in those in MMR but not MR4 (9 of 49 patients; 18.4%) (p<0.001). We now show in the Figure below that during the subsequent 12 months of complete treatment cessation in 117 stable MR4 patients, only 26 further recurrences and 4 withdrawals occurred, giving a recurrence-free survival (RFS) of 77% (90% CI: 71-83%) for the overall 24 months for this patient group. The recurrence rate on cessation is higher in the MMR but not MR4 group (20 recurrences and 4 withdrawals among 36 patients during cessation; 59% RFS overall; 90% CI: 29-52%); p=0.001). In both the stable MR4 group and the MMR but not MR4 groups, no difference in RFS was seen between patients in MR4.5 at entry and those not. In multivariable Cox proportional hazards modelling, addition of the baseline entry PCR result did not add to the predictive effect on RFS of the prior 12 month PCR pattern, whereas the duration of TKI treatment was an additional predictive factor (p=0.058; HR 0.93), in line with recent data from EUROSKI. The probability of RFS remains unrelated to age, gender, performance status or prior TKI (imatinib vs second generation). No progression to advanced phase was seen; one case lost haematological response.

S424
ASSESSMENT OF IMATINIB 400MG AS FIRST LINE TREATMENT OF CHRONIC MYELOID LEUKAEMIA: 10-YEAR SURVIVAL RESULTS OF THE RANDOMIZED CML STUDY IV
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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.

Methods: 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). Patients 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 all a1e1a2/a1e1a2 transcripts; Ph– patients and those with unknown Ph status were excluded from the mITT population. Safety was assessed in all patients (n=536) or in the intent-to-treat (mITT) population (47.2% vs 36.2%; P=0.02) as well as in the mITT 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=0.008), 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 adverse events (8.7% vs 4.6%) and increased alanine (19.0% vs 1.5%) and aspartate (9.7% vs 1.9%) aminotransferase levels 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.
 artisans: chronic myeloid leukemia patients were not different in molecular relapse after stopping imatinib in mr4 whether relapse was detected or not - when adjusting for number of control transcripts

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background: with imatinib (im), most patients with chronic myeloid leukemia (cml) achieve deep molecular responses. six months after stopping tyrosine kinase inhibitor in deep response in the euro-ski trial, 61% of the patients were in mr4, suggesting a survival advantage and in major molecular remission (3-log reduction in bcr-abl1 levels) (mahon ash 2016). between patients with and without bcr-abl1, the difference in rfs at 6 months was not significant when assessing bcr-abl1 detectability at the mr4.5 level (at least a 4.5-log reduction in bcr-abl1) (pfirrmann ash 2016).

aims: for 91 of 448 patients of the euro-ski learning sample, the sensitivity to claim undetectable disease at the mr4.5 level was not given. aim was to investigate whether rfs probabilities would be different when comparing detectable and undetectable disease at the mr4 level.

methods: detectability of bcr-abl1 depends on the number of control gene transcripts. to reduce bias when comparing “mr4 detectable disease” (mr4 but still detectable bcr-abl1 transcripts; i.e. 0.01- 0.0033% is) and “mr4 undetectable disease” (mr4 without detectable bcr-abl1; based on 10.000-31.999 abl1 or 24.000-76.999 gus8 copies), two samples with similar sensitivity of identifying bcr-abl1 were to be identified using propensity scoring (ps) matching (rosenbaum, rubin 1983). apart from type (abl1 or gus8) and number of control gene transcripts, matching variables were interferon alpha treatment, sex, age, and in major molecular remission (3-log reduction in bcr-abl1 levels) (mahon ash 2016).

results: a total of 448 patients had eligible, complete, and sufficient molecular data prior to and within the first 6 months after stopping im treatment. all molecular results had sensitivity at the mr4 level with yet detectable disease in 196 patients (44%). with small differences in gus8 copy numbers (used in 96 of 448 cases, u test (detectable vs undetectable): p<0.5), prior to ps matching, median numbers of abl1 transcripts were higher with mr4 detectable disease (78,975 vs 68,925 with undetectable disease; p=0.0511, not significant (n.s.)). at 6 months, patients with detectable disease again had 52% (95% confidence interval (ci): 44-60%) rfs probability, and patients with undetectable disease 63% (ci: 58-69%). relapse was significantly higher in patients with detectable disease (odds ratio: 1.63: ci: 1.096-2.343). ps matching resulted in 173 patients per group. median numbers of abl1 transcripts changed to 82,142 (detectable) and 75,750 (undetectable disease; n.s.). at 6 months, patients with detectable disease again had 52% (45-59%) rfs probability, and patients with undetectable disease 59% (52-66%). in the logistic model stratified for the matched pairs, for relapse at 6 months, the odds ratio for mr4 with detectable to undetectable disease was 1.308 (ci: 0.862-1.948, n.s.).

summary: using the mr4 threshold, after matching on number of control transcripts and other factors, results suggest little or no impact of detectability of bcr-abl1 on rfs. time in deep response seems to be more important. in daily routine, many labs produce reliable outcome at the mr4 but not always at the mr4.5 level. discontinuation at the mr4 level, irrespective of detectability of bcr-abl1 residual disease, appears safe, with a good chance of success when performed as in euro-ski. with ps matching, bias and differences but also power was reduced. to judge whether molecular response on the mr4 level is sufficient, further data is welcome.
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 complex may promote AML. Reversing those changes could represent a novel therapeutic approach.

Aims: Gain of chr2q22 is the most common focal amplification in complex karyotype AML. HMGN1 is a chromatin-regulatory protein on 2q22 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 than controls and remained as immature myeloblasts (84% CD11b+Gr1+ in WT vs 4% in OE-HMGN1, p<0.002; Fig A). Gene set enrichment analysis revealed more similarity to undifferentiated hematopoiesis and leukemia signatures in OE-HMGN1 cells. MINT-ChIP indicated higher global and locus-specific levels of H3K27ac in OE-HMGN1 cells (Fig B, upper panel), consistent with an increase in gene transactivation confirmed by RNA-seq. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, high-expressing Cd11b+Gr1+ in WT vs 4% in OE-HMGN1, p<0.001 (Fig A). In agreement with gene expression, among the most differentially measured histone peaks genome-wide were higher H3K27ac at HoxA genes promoters at all differentiation time points analyzed (Fig B, lower panel). Competitive transplantation demonstrated an advantage to OE-HMGN1 stem and progenitor cells. The clonal dominance of OE-HMGN1 over WT cells extended to all populations analyzed (long- and short-term HSCs, multipotent progenitors, CMP, GMP and MEP; Fig C) and to mature lineages (myeloid, B and T cells). MINT-ChIP indicated only higher levels and progenitors were more expressed as an increase in H3K27ac at peak cell cycle and leukemia-related genes in the context of OE-HMGN1, H3K27 acetylation is catalyzed by the CBP/p300 histone acetyltransferase (HAT), suggesting that HAT inhibition could target leukemias with HMGN1 overexpression. Indeed, treatment of myeloid progenitors with the CBP/p300 inhibitor C646 rescued the differentiation block in OE-HMGN1 cells (93% CD11b+Gr1+ in WT vs 80% in OE-HMGN1, p<NS).

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.

S429

PIWIL4 ACTS AS A PIRNA BINDING, EPIGENETICALLY ACTIVE AND GROWTH REGULATORY PROTEIN IN HUMAN ACUTE MYELOID LEUKAEMIA

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Background: Piwi proteins are critically important for maintaining the self-renewing stem cell population in lower organisms through epigenetic silencing of transposable elements via DNA methylation and H3K9me3 marks, in close interaction with a novel class of non-coding RNA called piwi interacting RNA (piRNA).

Aims: There are neither precise data on the function of Piwi proteins in human acute myeloid leukemia (AML), nor are there reports on expression of piRNAs in this disease. We employed functional techniques and NGS to understand the role of human PIWI-like protein, PIWIL4 and its associated piRNA in AML.

Methods: We assessed the expression of human PIWil genes in AML and healthy bone marrow cells using qRT-PCR. Murine stem progenitors were transduced with AML specific oncogenes to evaluate the effect on PIWIL expression. Short RNA mediated knockdown (KD) of PIWIL was performed on AML cell lines, AML patient bone marrow (BM) and healthy cord blood CD34+ stem progenitors and the impact on growth was determined in vitro and in vivo assays. Western blot, ChIP-seq for H3K9me3 and RNA-seq were performed to assess the impact of PIWIL KD on the epigenetic landscape. In this study, we assessed the transcriptome of the AML cell line THP-1, PIWIL4 knockdown by LC-MS was performed to determine the binding partners of PIWIL4. PAR-CLIP and microarray were performed to identify piRNAs that physically bind to PIWIL4 and to test the impact of PIWIL4 KD on piRNA expression.

Results: Among the family of human PIWIL genes, PIWIL4 showed the highest expression level and was ubiquitously expressed in healthy hematopoietic stem/progenitors, mature lymphoid and myeloid cells. Importantly, PIWIL4 was aberrantly higher expressed in more than 89% of the AML patients (n=68; p<0.0001) compared to normal CD34+ BM and total BM cells (n=3). Overexpression of AML specific oncogenes in murine stem progenitors, within 96h post-transduction, induced a 6 to 8 fold increase in PIWIL expression compared to GFP control (n<0.0001). Knockdown (KD) of PIWIL4 in AML cell lines significantly impaired proliferation and clonogenic growth in vitro (n=3; p<0.0001) and delayed onset of leukemia in NSG mice (n=8; p<0.0001). PIWIL4 KD in primary AML patient BM cells lead to 5-fold decrease in clonogenicity (n=3, p<0.001), but had no impact on clonogenicity of healthy stem progenitors in vitro (n=4). Western blot and ChIP-seq (n=2, MACS1.4, p<0.01, FDR<0.01) in THP-1 cell line revealed a marked global reduction in repressive H3K9me3 marks upon PIWIL4 KD. Over 500 promoter and 600 gene body associated loci exhibited loss of H3K9me3 marks. RNA-seq analyses revealed 4000 differentially expressed genes and PIWIL4 depletion. 30% of the loci that lost H3K9me3 marks at promoters and gene body were differentially expressed in RNA-seq (folds>0.05, adj. p<0.01). These genes belonged to pathways associated with RNA metabolism, transcription and cell death. Moreover, these genes were enriched for binding sites of SETDB1, an H3K9me3 establishing histone methyltransferase (ENRICHCR, p<0.01, FDR<0.01). Notably, using IP/LC-MS, PIWIL4 was found to associate with SETDB1 in 293T cells. 560 unique piRNAs were found to physically bind to PIWIL4 and 981 unique piRNAs were differentially expressed upon PIWIL4 depletion in THP-1 cells.

Summary/Conclusions: Thus, collectively, we could show for the first time that PIWIL4 expression is deregulated in human AML and acts as a piRNA binding, epigenetically active growth regulatory protein in human AML.

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METTL3 CONTROLS TRANSLATION OF TARGET MRNAs BY N6 METHYLATION OF ADENOSINE RESIDUES IN THEIR CODING SEQUENCE AND CONSTITUTES A NOVEL THERAPEUTIC VULNERABILITY OF ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) patient survival remains below 30% and there have been no major new anti-AML therapies for decades.
Figure 1.

Summary/Conclusions: Our results show that METTL3 controls translation of specific mRNAs by binding their TSS and introducing m6A at [GAG]n motifs within their CDS, in turn increasing their TE. These mRNAs code for proteins essential for AML cell survival, making METTL3 a novel therapeutic vulnerability of AML.

Acquired and inherited platelet disorders

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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 retinoid 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 T cell function in patients with thrombocytopenia (Towsley DM et al. Blood 2016). The combination of ATRA and danazol may work synergistically based on the mechanism of action targeting both increased platelet destruction and insufficient platelet production.

Aims: To investigate the efficacy and safety of ATRA plus danazol in patients with corticosteroid-resistant or relapsed ITP.

Methods: A multicentre prospective study was performed in non-splenectomized corticosteroid resistant/relapsed ITP patients. Participants were at least 18 years of age, had a platelet count of less than 30×10^9/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: From 2012 to 2016, 130 consecutive patients were enrolled from 5 different tertiary medical centres in China. Thirty-seven patients were ineligible and excluded, leaving 93 patients randomized to the ATRA+danazol group (n=45) and the danazol group (n=48). At 12 months' follow-up, sustained partial or complete response was achieved in 71.6% of patients in the danazol ATRA group, significantly higher than 47.2% for danazol monotherapy (p<0.001). Additionally, 92.5% and 42.5% of patients receiving ATRA+danazol achieved at least one response (R), while only 58.3% and 11.1% of patients with danazol monotherapy achieved R and CR, respectively. In patients achieving CR or R, the median time to treatment response was 30.5 days with a peak platelet count of 155×10^9/L in the danazol+ATRA group compared with 49 days with a peak PLT of 69×10^9/L in the danazol group. Multivariate analysis revealed that the initial response at day 28 and the median ITP duration were the potential variables associated with a sustained response. There was no treatment-related death due to adverse events. One patient receiving danazol monotherapy died from intracranial haemorrhage 4 weeks after study enrollment.

Summary/Conclusions: Our findings demonstrate that the combination of ATRA and danazol is safe and effective in achieving a rapid and long-lasting response, making it a potential promising therapeutic option for patients with corticosteroid-resistant or relapsed ITP. This study is registered at www.clinicaltrials.gov as # NCT01667263.

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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
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MONOA LLEOSS-OF-F UCTION-M UTATION IN THE THROMBOPET IN (THPO) GENE IS RESPONSIBLE FOR A NEW FORM OF INHERITED THROMBOCYTOPENIA

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Background: The THPO-MLP axis plays a central role in platelet biogenesis: it activates the signaling cascade inducing megakaryocytes (MKs) differentiation from progenitor cells and regulates MK maturation, proplatelet extension, and nascent platelets release into the bloodstream. Different diseases are known for this genetic abnormality, including thrombocytopathy and THPO. Gain-of-function mutations in both genes cause congenital thrombocytopenia, while loss-of-function mutations in MPL result in congenital amegakaryocytic thrombocytopenia. Patients affected by this form of inherited thrombocytopenia (IT) present at birth with isolated thrombocytopenia, which always evolves into severe bone marrow aplasia. Similarly, a homozygous loss-of-function variant in THPO gene was found to be responsible for recessive aplastic anemia in a Monacianese family.

Aims: To unravel the molecular basis of ITs and to improve the clinical and laboratory diagnosis of patients affected with the new IT.

Methods: Whole exome sequencing (WES) was performed in 86 propositi with an unknown IT. They were part of our case series of 274 consecutive families, 151 of which remained without a definite diagnosis at the end of the diagnostic workup carried out according to the diagnostic algorithm proposed in 2003 by the Italian platelet study group and subsequently updated to include the most recent discovered disorders (Clin Genet 2016;89:141). The investigation was approved by the Institutional Review Board of the IRCCS Policlinico San Matteo Foundation and all patients gave written informed consent.

Results: WES in 86 propositi with unknown IT identified 2 unrelated individuals (Family A and B) carrying the heterozygous variant c.724T>G in Arg31 which is expected to result in a mutant protein degradation and THPO haploinsufficiency. In each family the segregation with the disorder was confirmed analyzing one affected relative. Bleeding tendency was absent in all cases. All patients had mild thrombocytopenia; blood film examination did not identify any morphological traits characteristic of MKs expanded megakaryocytes with slightly increased size in patients of family A. In vitro platelet aggregation and surface expression of GPIb/IIa and GPIb/IX were investigated in the two patients of Family B and gave normal results. The mild severity of thrombocytopenia and the absence of qualitative platelet defects, at least in the two subjects of family B, are consistent with the absence of bleeding tendency in affected subjects. THPO serum level was at the lower limit of the normal range in the two subjects of family B, the only available for this assay. This result was in agreement with our hypothesis that THPO mutations were expected to result in haploinsufficiency.

Summary/Conclusions: The Arg31 mutation in THPO causes a new autosomal dominant form of mild, non-syndromic thrombocytopenia. This innocuous disorder is relatively rare (1.3% of families of our case series) but it has to be distinguished from the more severe autosomal dominant ITs with normal platelet size deriving from mutations in ETV6, ANKR2D and RUNX1, since they predispose to the development of hematological malignancies. Because of the similarity of the clinical features and the lack of reliable laboratory markers, we suggest to perform genetic analysis in all subjects with autosomal dominant thrombocytopenia and normal platelet size in order to identify their disorders, define prognosis and organize an appropriate follow-up regimen.

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POSITION OF THE GFIB1 ZINC FINGER MUTATION DECOUPLES CD34 EXPRESSION FROM ALPHA-GRANULE DEFICIENCY IN GFIB1-RELATED PLATELET DISORDERS

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Background: GFIB1 is a transcription factor that plays an important role in haematopoiesis. Families with a mutation of the fifth DNA-binding zinc-finger domain of GFIB1 experience bleeding and have a platelet phenotype characterised by macrothrombocytopenia, increased CD34 expression and alpha-granule deficiency.

Aims: To explore the function of other zinc finger domains of GFIB1 we have characterised two unrelated families with a GFIB1 variant, C168F, predicted to disrupt the first zinc-finger domain and compared the phenotype with a previously described pedigree with the H249fs mutation that disrupts the fifth zinc-finger domain.

Methods: Clinical platelet phenotypes were determined by light and transmission electron microscopy and functional studies performed by light transmission and whole blood impedance aggregometry. Platelet protein expression was measured by flow cytometry and western blotting. DNA-binding of variants was determined by gel mobility shift assays (EMSA) and changes in gene transcription by luciferase assays. Cellular phenotypes were then studied in patient specific iPSC derived megakaryocytes.

Results: Megakaryocytes of individuals with C168F are thrombocytopenic (mean platelet count =107 x10^9/μL, n=8) but lack the collagen induced aggregation defects and bleeding symptoms observed in individuals with H249fs (ISTH BAT, P<0.01). Alpha granule content observed by microscopy and quantitated by western blotting of granule related proteins, P-selectin and fibrinogen, were similar between C168F and control platelets and this was significantly greater than that observed for the H249fs mutation (P<0.01). EMSA studies indicate that the C168F variant retails the ability to bind DNA whereas the H249fs mutation altering Zn finger 5 abrogates DNA binding. Despite retaining the ability to bind DNA, the C168F variant de-represses gene transcription at 7UB1, TUBB1 or MYH9 compared to C168F or MYH9.

Summary/Conclusions: The C168F mutation is more severe than that observed with the non-DNA-binding H294fs mutation (P<0.01). The transcriptional de-repression observed at the CD34 promoter with both Zn finger 1 and 5 variants was validated by an increase in platelet surface CD34 measured by flow cytometry and total CD34 protein measured by western. The absence of expression at young-mid life although expected in the CD34 promoter with GFIB1 mutation as increased CD34 expression was not observed in platelets derived from individuals with FL11, RUNX1 or MYH9 mutation. To validate these clinical observations, iPSCs were generated from the different pedigrees and megakaryocyte differentiation performed in vitro. Megakaryocyte CD34 expression was increased in cells derived from individuals with both C168F and H294fs variants but alpha granule deficiency was only observed in cells containing the non-DNA-binding H294fs mutation.

Summary/Conclusions: Mutations altering GFIB1 Zn finger 1 cause thrombocytopenia with increased CD34 expression but these platelets retain alpha-granule deficiency. Whole exome sequencing identified a second mutation, C168F, which disrupts zinc-finger domain 1 and localizes to a critical region for Zn-finger domain specificity. Mutations in the Zn-finger domains of GFIB1 are associated with clinical observations, iPSCs were generated from the different pedigrees and megakaryocyte differentiation performed in vitro. Megakaryocyte CD34 expression was increased in cells derived from individuals with both C168F and H294fs variants but alpha granule deficiency was only observed in cells containing the non-DNA-binding H294fs mutation.

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TREATMENT OF PRIMARY ADULT CHRONIC IMMUNE THROMBOCYTOPENIA (CITP) WITH FOSTAMATINIB, AN ORAL SYK INHIBITOR: RESULTS OF TWO RANDOMIZED, PLACEBO-CONTROLLED PHASE 3 STUDIES

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Background: ITP is characterized by autoantibody-directed platelet destruction mediated by activated monocyte Fc receptors which signal via spleen tyrosine kinase (tok). A Phase 2 trial of the oral syk inhibitor Fostamatinib (FOSTA) in 16 patients (pts) with refractory ITP provided preliminary efficacy and safety data (Podolanczuk et al., 2009).

Aims: To evaluate the efficacy and safety of FOSTA in adult cITP in 2 parallel, identical, multi-center, randomized, double-blind phase 3 studies (S047 and S048) of 24 weeks duration, followed by an open label study (S049).

Methods: 150 pts with 3 platelet (plt) counts (ct) <30K/μL were enrolled (76 in S047, 74 in S048) with 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 (20-88); 93% were Caucasian; 93% had cITP; median disease duration: 8.5 y; median baseline plt ct: 16K/μL. Prior therapies received by pts included 94% steroids, 47% TPO-RAs, 35% splenectomy, and 32% rituximab. Stable response (SR) was defined as a plt ct ≥50K/μL at 4 of 6 biweekly visits over Weeks 14-24; intermediate response (IR) as at least 2 consecutive bi-weekly plt cts ≥50K/μL, both without rescue AE. Successive plt increases ≥20K/μL were considered partial response (PR). Serious bleeding occurred in 5.6% of the NR and 10.2% of placebo pts. 14/49 (29%) placebo pts had a plt increase ≥20K/μL (p=0.005). Three of 18 pts with SR and IR, median time to first plt ct ≥50K/μL was 2 weeks. Age (< or ≥65 y), gender, baseline plt ct <15K/µL, prior TPO-RA or splenectomy did not substantially affect response. In S049, 54/101 (54%) FOSTA pts and 52/101 (51%) placebo pts achieved an IR, making the overall response rate 29% (29/101) for FOSTA vs 2% (1/49) for placebo (p=0.0001). The median plt cts were 95K, 49K, 20.5K and 17.5K/μL in SR, IR, non-responders (NR) and placebo pts, respectively. In SR and IR, median time to first plt ct ≥50K/μL was 2 weeks. Age (< or ≥65 y), gender, baseline plt ct <15K/μL, prior TPO-RA or splenectomy did not substantially affect response. In S049, 9/44 (21%) pts newly treated with FOSTA have a SR, consistent with S047 and S048. Fifty-four of 101 (54%) FOSTA pts and 49/104 (47%) placebo pts had a plt increase ≥20K/μL (p=0.005). Three of 18 (17%) SR and 1/11 (9%) IR to FOSTA compared to 26/72 (36%) NR and 22/49 (45%) of the placebo group received 21 rescue medication, respectively. In S047-S048, serious bleeding occurred in 5.6% of the NR and 10.2% of placebo pts, but in not the 29 responders. The number of pts with ≥1 adverse event (AE) was similar in FOSTA vs placebo (83% vs 75%). 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 cts in certain pts with heavily pre-treated, severe cITP of long disease duration. AEs are mostly mild or moderate in severity. Given its unique mechanism of action based on inhibition of syk, FOSTA could, if approved, be an important alternative as single agent and be a useful component of combination therapy for pts with difficult cITP.

References

Acute lymphoblastic leukemia - Biology

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The TING 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 (ALL)


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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 related 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 revealed lesions affecting known driver genes in the samples. In 80% of the patients we detected activating mutations in genes whose protein products are involved in signaling, including receptors (CRLF2, IL7R, FLT3), or downstream effector enzymes (JAK1/2, KRAS and NRAS). In contrast to a previous report, we observed that lesions in CRLF2 are early events during DS-ALL evolution, as it is evident by its high allelic frequency, and are main- tained at relapse. The genetic make-up differed significantly between these two major subtypes of DS-ALLs. CRLF2neg DSALLs were characterized by enhanced RAS signaling coupled by mutations in chromatin remodeling genes, in particular CREBBP. In contrast CRLF2pos DS-ALLs were characterized by high dynamics of proliferative signaling. At diagnosis CRLF2 rearrangements were almost always combined with secondary activating sig- naling events in JAK-STAT pathway suggesting that signaling is driving the development of these leukemias. However JAK2 mutations were often lost at relapse and replaced by clones with mutated RAS. Thus the presence of JAK2 activating mutations at the time of diagnosis are associated with sensi- tivity to upfront chemotherapy. Surprisingly we discovered loss-of-function mutations or deletions of USP9X, a deubiquitinase previously described as an oncogene that positively regulates JAK2 signaling, in 25% of CRLF2pos ALLs, in both our study and published data. We therefore tested the coun- terintuitive hypothesis that loss of a positive regulator of JAK2 enhances the fitness of JAK2-STAT driven JAK2 mutated leukemic cells. Both pharmacological and genetic CRISPR-mediated loss of USP9X reduced STAT5 phosphorylation and enhanced the survival of CRLF2-JAK2R863Gs transduced ALL cells. To test directly the effect of JAK inhibition, we treated CRLF2/JAK2R863G transduced cells with increasing doses of ruxolitinib, a 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-JAK2R863Gs expressing ALL cells.

Summary/Conclusions: These observations suggest that genetic or pharma- cological 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 the secondary activating effect of JAK2 specific inhibitors may be limited. Rather, combined signaling 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 genetically crossed cMyb knockin mice with Pten conditional knockout mice, to study the 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-Cre+/−, R26-Myb+/− 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-cell leukemia/lymphoma with a median latency of 17 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A).

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.

Figure 1.

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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 genomic duplications of the MYB locus itself. Recently, a new genetic mechanism for the generation of oncogenic super-enhancers in malignant T cells was identified, and suggests a general role for MYB in the regulation of T-cell specific super-enhancer activity.

Aims: We want to identify the role of enhanced MYB activity in super-enhancer driven oncogenic transcription in the context of malignant T-cell development and investigate the in vivo role of cMyb in the initiation and maintenance of T-ALL.

Methods: To evaluate if cMyb could act as a bona fide oncogene in the pathogenesis of T-ALL, we developed a conditional R26-driven cMyb overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the cMyb gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).

Results: Here, we report a novel conditional Myb knockin mouse model (R26-Myb). To study the in vivo oncogenic capacity of Myb, we initially crossed this conditional Myb knockin model with VavCre mice, in order to obtain hematopoietic specific expression of Myb and the EGFP/luciferase from the ROSA26-promoter. Notably, Vav-Cre+/−, R26-Myb+/− mice developed T-cell lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-ALL, and T-cell specific deletion of Pten (using Lck-Cre) results in T-cell leukemia/lymphoma development with an average of 17 weeks. Using this strategy, we obtained mice that overexpress R26-driven cMyb and lack Pten in developing T-cells and found that cMyb expression synergizes with Pten deletion, resulting in fully penetrant and accelerated T-ALL formation (median survival of 84 days instead of 118; p = 0.0003; Figure 1B). Finally, we used this novel murine T-ALL model to identify new therapeutic strategies for MYB dependent T-ALL. Importantly, the tumor cells from the cMyb knockin mice are luciferase-positive and are therefore suitable for in vivo drug testing using bio-luminescence. 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.

Figure 1.

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.
THE T-CELL LEUKEMIA ASSOCIATED RIBOSOMAL RPL10 R98S MUTATION ENHANCES JAK-STAT SIGNALING

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Background: Several somatic ribosome defects have recently been discovered in cancer, yet their underlying oncogenic mechanisms remain poorly understood. Alterations in ribosomal protein genes RPL5, RPL10, and RPL22 have been described in ~20% of T-cell acute lymphoblastic leukemia (T-ALL) cases. Whereas RPL5 and RPL22 show heterozygous inactivating mutations and deletions, RPL10 contains a clear mutational hotspot at residue arginine 98 (R98), with 8% of pediatric T-ALL patients harboring this RPL10 R98S missense mutation.

Aims: Investigating the pathogenic role of the recurrent R98S mutation in ribosomal protein L10 (RPL10) in T-ALL.

Methods: A label-free quantitative proteomics experiment was performed to screen for differentially expressed proteins in engineered mouse lymphoid BarF3 cells expressing RPL10 WT or RPL10 R98S. Differences in protein expression were further validated in hematopoietic cells derived from a transgenic RPL10 R98S knock-in mouse model and in material derived from xenografted T-ALL patient samples.

Results: The differential proteome screen revealed overexpression of several Jak-Stat signaling components (Csf2rb/2, Jak1, Stat1, Stat3, Stat5a/b and Stat6) in engineered RPL10 R98S mouse lymphoid cells, which we confirmed in hematopoietic cells derived from a transgenic RPL10 R98S 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 human Jak-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 proteasome 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 proteasome activity and enhanced sensitivity to the clinically used proteasome 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 proteasome inhibitors.
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 chemotherapeutic agents with the associated risk for thrombocytopenia. However, the consequences of a recurrent VTE or a major bleeding event (MB) might be different in terms of mortality. As a result, the risk of VTE recurrence or a MB event might bear different weights. A previous systematic review has suggested that the case fatality rates of VTE recurrence and MB are similar. However, heterogeneity in study design, outcomes and in particular the types of populations included, limited the interpretation and applicability of the results. Clinical decision making uses estimations of risk and benefit for any given intervention. In the case of VTE, anticoagulants are the cornerstone of treatment having a proven benefit in reducing the risk of recurrent VTE events with an associated increase in the risk of bleeding. Therefore, determining the risk-benefit of anticoagulants might allow for better informed treatment decisions, in particular in a population at high risk for both ends of the spectrum. Therefore, herein we sought to estimate the risk and benefit of anticoagulant therapy in cancer patients developing a VTE using data from administrative databases.

Aims: We investigated 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 previously validated algorithm 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 identified 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 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). 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: Actual guidelines recommend Padua and Khorana score for thromboembolic (TE) risk estimation for cancer patients in general. These existing models are quite limited for designation of lymphoma patients for TE events, as their development is not based on features specific for hematological patients.

Aims: The aim of this study was to compare diagnostic performance of these suggested predictive models, as well as Thrombosis lymphoma (Thylo) score, developed by our group, which is more specific for lymphoma patients.

Methods: The study population included all consecutive patients with a confirmed diagnosis of non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), and chronic lymphocytic leukemia (CLL) who were treated in the Lymphoma Departments of Clinical Center Serbia and Clinical Center Kragujevac in period from 2006 to 2014. Data for newly diagnosed and relapsed patients who had completed a minimum of one chemotherapy cycle were prospectively collected for all venous and arterial TE events from time of diagnosis to 3 months after the last cycle of therapy. Data for specific thromboembolic, clinical, and laboratory markers were recorded. TE events were defined as venous thrombosis (VT), arterial thrombosis (AT), and major bleeding events (MB).

Aims: The aim of this study was to estimate the risk of thrombosis of 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 embolism, transient ischemic attack, angina pectoris, myocardial infarction, and arterial embolism and thrombosis.

Results: NHL patients had a statistically significant increase in risk of any type of thrombosis compared to controls (HR: 1.58; 95% CI: 1.53-1.62). The risk was significantly increased for all three types of thrombosis: deep vein thrombosis (HR: 2.29; 95% CI: 2.11-2.48), pulmonary embolism (HR: 1.15; 95% CI: 1.07-1.24) and arterial thrombosis (HR: 1.20; 95% CI: 1.16-1.23). The risk of thrombosis did not change during the study period for the NHL patients. There was an increased risk of thrombosis for NHL patients when compared to controls and to study time trends in the risk of thromboembolism.

Aims: The incidence of thrombosis for NHL patients started to increase about five months before the diagnosis of NHL, and reached its peak a month before diagnosis. The incidence stayed increased for the first year after diagnosis.

Summary/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.
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 LMHW was not prescribed. The main outcomes were: VTE (confirmed by objective methods), clinically related and 30 days after discharge. Descriptive statistical analysis and correlation studies are required.

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.

IDENTIFICATION OF A NEW AND RELATIVELY FREQUENT SERPINC1 GENE DEFECT CAUSING ANTIITHROMBIN 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

### Table 1. Clinical features in Group1, Group 2 and both.

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Age</th>
<th>Mean Age</th>
<th>Advanced VTE (%)</th>
<th>Complete Prophylaxis (%)</th>
<th>C. Rel. 30d (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>54.1</td>
<td>0.21</td>
<td>0.36</td>
<td>0.23</td>
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<tr>
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<td>54.1</td>
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<tr>
<td>Both</td>
<td>50</td>
<td>54.1</td>
<td>0.21</td>
<td>0.36</td>
<td>0.23</td>
</tr>
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#### Note

- **Group 1:** Patients with new diagnosis
- **Group 2:** Patients with relapse
- **C. Rel. 30d:** Clinical related events within 30 days after discharge
- **Advanced VTE:** VTE confirmed by objective methods
- **Complete Prophylaxis:** Prophylaxis used within 24 hours after admission

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**Summary/Conclusions:** The new e-alert system further increases the use of VTE prophylaxis in hospitalised cancer patients, although this was not associated with a reduction in the VTE incidence. A relevant number of VTE events occur despite prophylaxis with standard LMWH. Identification of risk factors for thromboprophylaxis failure is needed.

This work has been funded by a biomedical research grant with the Laboratory of Pharmaceutical ROVI and an aid to the research projects of the Instituto de Salud Carlos III and the FEDER (PI13/01029).
with no apparent gene defect by either Sanger sequencing of 7 exons or by NGS analysis of the whole gene using the Ion Torrent platform, revealed a 193 bp insertion, which corresponded to a tandem duplication involving exon 6. Family studies revealed the same duplication in 5 relatives, all with AT deficiency (60-75%). The first MLPA analysis of this case failed to detect the duplication and only after a fine readjustment, it was detected. MLPA analysis under the new conditions of the remaining 59 cases with unknown molecular base for their AT deficiency identified one additional case, P2, with potential duplication of exon 6. P2 was a 17 year-old female with 41% of anti-FXa activity, who developed deep venous thrombosis. Sanger and NGS sequencing also failed to detect any genetic defect in P2. A set of primers specific to detect tandem duplications of exon 6 was designed with forward primer from 3' end of exon 6, and reverse primer from 5' of exon 6. This set of primers only rendered amplification in the two cases with exon 6 duplication. The 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. We also developed a simple and specific method to detect duplications in tandem of exon 6.

Stem cell transplantation - Experimental

S446

CYTOSOLIC NUCLEIC ACID SENSORS PROMOTE INTESTINAL EPITHELIAL INTEGRITY DURING ACUTE TISSUE DAMAGE AND PROTECT FROM GRAFT-VERSUS-HOST DISEASE

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Background: The epithelial lining of the gastrointestinal (GI) tract represents the first line of defense mediating protection from microbial challenge. Next to producing antimicrobial molecules, Paneth cells contribute to this defense by providing a supportive niche for intestinal stem cells (ISCs) maintaining the epithelium. Loss of intestinal barrier function by total body irradiation (TBI) or chemotherapy (CTx) is an essential step in enhancing the development of intestinal graft-versus-host disease (GVHD). Moreover, low-grade TKI reactivation may lead to IFN-I-dependent intestinal organoid growth. Consistent with this, STING-dependent 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 promote epithelial integrity and could be exploit-
ed interventionaly to promote intestinal barrier function and prevent GVHD.

Aims: We aimed at characterizing the role of RIG-I-MAVS and STING during allo-HSCT, and at understanding mechanisms by which activation of these pathways can promote barrier function to enhance healing after genotoxic tis-
sue damage.

Methods: We used an integrated approach with pathophysiologic mechanistic studies on IECs in experimental mouse models (MHC-mismatched and minor histocompatibility antigen (mHLA)-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 func-
tion (organoid cultures, barrier function test)

Results: Mice lacking MAVS were more sensitive to total body irradiation (TBI)- and chemotherapy induced intestinal barrier damage, and, like RIG-I-deficient mice, developed severe 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 Regllity. 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.

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CD4 T CELLS RECOGNIZING MISMATCHED HLA-DP AFTER ALLOGENIC STEM CELL TRANSPLANTATION SHOW TISSUE SPECIFIC REACTIVITIES

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Background: Expression of HLA class II molecules is under non-inflammatory conditions predominantly restricted to hematopoietic cells. However, donor CD4 T cells directed against mismatched HLA-DP can cause Graft-versus-Host Dis-
ease (GVHD) after allogeneic stem cell transplantation (alloSCT) or donor lympho-
phyte infusions from HLA 10/10 matched but HLA-DP mismatched donors due to upregulation of HLA class II expression under inflammatory conditions. It is often assumed that allo-HLA-DP directed CD4 T cells recognizing peptides encoded by household genes presented in foreign HLA-DP and that every cell that expresses the mismatched HLA-DP allele is a target for these T cells. However, in vitro experiments illustrated that allo-HLA-DP directed CD4 T cells were not always recognizing patient derived fibroblasts induced to express HLA-DP. We hypothe-
sized that HLA-DP directed CD4 T cells can have tissue specificity if the presented peptides in HLA-DP are encoded by genes with tissue specific expression.
Aims: The aim of the study is to investigate whether donor CD4 T cells recognizing mismatched HLA-DP show tissue specific reactivities.

Methods: In a randomized clinical trial we treat patients 3 months after T cell depleted alloSCT from HLA 10/10 matched, HLA-DP mismatched, donors with 0.25-0.50 x 10^6/kg donor CD4 T cells to promote immune reconstitution. In 4 patients, Graft-versus-Leukemia reactivity and/or organ specific GVHD occurred after the infusion. To characterize the immune responses in these patients, in vivo activated T cells were clonedally 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*04:01 reactive T cell clones were found from patient 3, of which 6 recognized only hematopoietic target cells, whereas other clones again showed various tissue specificities. From patient 4, 26 HLA-DPB1*01:01 reactive T cells could be isolated which all recognized biliary epithelial cells with or without co-recognition of other target cells. In addition, also 11 HLA-DPB1*03:01 reactive T cells were isolated, again with different tissue specificities.

Summary/Conclusions: These results illustrate that donor CD4 T cells directed against mismatched HLA-DP show differential recognition of target cells including restricted specificity for cells of hematopoietic origin. Donor CD4 T cells recognizing hematopoietic target antigens in the context of patient specific HLA-DP alleles can be used to mediate tumor specific immune responses after HLA 10/10 matched unrelated stem cell transplantation.

S449 ABERANT T CELL RESPONSES IN THE BONE MARROW MICROENVIRONMENT OF PATIENTS WITH POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

MESENCHYMAL STROMAL CELLS STIMULATE THE PROLIFERATION AND IL-22 PRODUCTION BY INDOMETHACIN STIMULATED INTRINSIC INNATE LYMPHOCYTES (ILC3S)

Background: We recently reported that relatively high frequencies of activated type 3 innate lymphoid cells (ILC3s) before and/or after ASCT were associated with a lower risk to develop GvHD, which may be related to the production of tissue-protective IL-22 by ILC3s.

Aims: To investigate if ILC3s can contribute to the therapeutic effect of MSCs, we studied the interaction between MSCs and ILC3s in vitro.

Methods: ILC3s isolated from human tonsils were CellTrace-labeled and cocultured with bone-marrow derived MSCs for 5 days in the presence of IL-2.

Results: Co-culture with MSCs significantly enhanced the proliferation of ILC3s and their IL-22 production. Reciprocally, ILC3s promoted ICAM-1 and VCAM-1 expression on MSCs. To assess the MSC effects in vivo, we created a mouse model for chronic GVHD and studied the interaction using ILC3s from GVHD patients. The interaction between ILC3s and ASCs in the BM resulted in higher percentages 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.

S450 HIGHER FREQUENCY OF SWITCHED MEMORY B CELLS PREDICTS THE INCIDENCE OF CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

Background: A discordant T cell response between the recipient and the Graft versus Host Cells (GVHC) may influence the development of chronic Graft versus Host Disease (cGvHD). This study aimed to analyze the frequency of switched memory B cells before allo-HSCT, to predict the incidence of cGvHD.

Methods: This prospective nested case-control study enrolled 20 patients with allo-HSCT matched with the donors (PGF) and 20 healthy donors (GD) after allo-HSCT, and 20 healthy donors (HD). Th17, Th1, Th1, Th2, Tc2 cells and regulatory T cells (Tregs) were involved in the pathogenesis of PGF to be evaluated. To investigate the incidence of chronic cGvHD, we compared the T cell subsets in the BM immune microenvironment, including Th1, Th1, Th1, Th2, Th2, Tc2 cells and regulatory T cells (Tregs), between patients with PGF and after allo-HSCT.

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 Th1 (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 Th2 (5.5% vs. 1.1%, P=0.0001) cells were markedly lower in the PGF group than in the GGF 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.
Methods: Peripheral blood (PB) samples were obtained from consented patients (pts) between day 80 and 110 (D100) after allo-SCT at The University of Texas MD Anderson Cancer Center from 2012 to 2015. Only pts who had not been diagnosed with cGVHD or progression of underlying malignancy by day 100 were eligible for this study. We analyzed CD19+CD20+ B cell subsets by flow cytometry. Subsets were defined as naïve (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- lato myelofib 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 acute GVHD had occurred in 45% of pts before D100. Thirty-six percent of pts were receiving steroids at D100. Forty-seven (59%) pts had detectable (≥0.1%) CD19+CD20+ signal on D100. In this subset, median B cell % was 3 (range 0.25-34) and median absolute number was 23 (1.8-419) x10³ cells /µL. Median % naïve, unswitched, and switched B cells was 89% (40.3-99%), 1.85% (0.3-8.5), and 2.1% (0-30), respectively. A total of 15 pts were diagnosed with cGVHD within 1 year after D100 including 11 with detectable B cells. ROC analysis did not identify predictive thresholds for overall B cell % or numbers. However, it identified predictive thresholds for each of the B cell subsets analyzed. The area under the curve (AUC) analysis indicated that % naïve (cutoff 97.3%), % switched (cutoff 4%) B cells were the most significant (AUC 86%) predictors of the incidence of cGVHD. Rounding up the cutoff values, we grouped pts into 3 mutually exclusive groups: 1) naïve B cells ≥90% (n=23, none had >5% switched B cells), 2) naïve B cells <90% and switched B cells ≤5% (n=11), and 3) naïve B cells <90% and switched B cells >5% (n=13). Patients (n=33) with undetectable B cells were considered together as one group. The rate of cGVHD after D100 was significantly higher in pts with <90% naïve and >5% switched B cells (HR=7, p<0.001) with a 1-year cumulative incidence of 61% (Figure). None of the characteristics listed above were significantly associated with the rate of cGVHD. Percent naïve and switched B cells did not correlate with receipt of steroids on D100. Patients with undetectable B cells were significantly more likely to have an underlying lymphoid vs myeloid malignancy (58% vs 33%, p=0.001); and those with ≥90% naïve B cells were significantly more likely to be ≤55 years of age at the time of allo-SCT (83% vs 42%, p=0.004).

Figure 1.

Summary/Conclusions: In conclusion, D100 frequency of naïve and switched B cells predicts the subsequent development of cGVHD. Lymphoid malignan- cies 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 cases of HSCT have been published. Thus, additional data are required to help in applying HSCT in PKD and guidelines are not available. To date, only four cases of HSCT have been published. Thus, additional data are required to help in applying HSCT in PKD and guidelines are not available.

Aims: The aim of this study was to make a worldwide inventory of all cases of PKD that have been treated by HSCT, and to evaluate indication, procedures employed, and outcome.

Methods: This is an international case series. Queries were send to national hematologists, and to experts in the field. 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 sixteenth patient showed mixed chimerism followed by spontaneous transition to full 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%. Seven 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 (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 transplantation, whereas only three out of eight (33.3%) patients older than ten survived. Patients younger than ten years old were less often splenectomized (p=0.001). All Asian patients (8/8) survived transplantation, whereas three out of eight European patients survived. Patients treated in Asian hospitals differed from European patients in that they were younger (p=0.001), less often splenectomized (p=0.041) and had a lower ferritin level prior to 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 patients. Due to the still relatively small number of cases no definite 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.
CRIZANLIZUMAB, A P-SELECTIN INHIBITOR, INCREASES THE LIKELIHOOD OF NOT EXPERIENCING A SICKLE-CELL-RELATED PAIN CRISIS WHILE ON TREATMENT: RESULTS FROM THE PHASE II SUSTAIN STUDY

Aims: This post-hoc analysis evaluated patients who did not experience a SCPC for the duration of the trial.

Methods: SUSTAIN was a randomized, double-blind, placebo-controlled, Phase II study (NCT01895361). Patients aged 16–65 years with SCD (including HbSS, HbSC, HbSPβ0-thalassemia, and HbSβ+–thalassemia genotypes) and 2–10 SCPC events in the previous 12 months were included. Concomitant use of hydroxyurea (HU) was permitted if the patient had been using it for ≥6 months and at a stable dose for ≥3 months. Patients were randomized 1:1:1 to receive intravenous crizanlizumab 5.0 mg/kg, 2.5 mg/kg or placebo. Loading doses were administered on days 1 and 15, followed by routine treatment every 4 weeks and at a stable dose for ≥3 months. Patients were randomized 1:1:1 to receive intravenous crizanlizumab 5.0 mg/kg, 2.5 mg/kg or placebo. Loading doses were administered on days 1 and 15, followed by routine treatment every 4 weeks and at a stable dose for ≥3 months. Patients were randomized 1:1:1 to receive intravenous crizanlizumab 5.0 mg/kg, 2.5 mg/kg or placebo. Loading doses were administered on days 1 and 15, followed by routine treatment every 4 weeks and at a stable dose for ≥3 months. Patients were randomized 1:1:1 to receive intravenous crizanlizumab 5.0 mg/kg, 2.5 mg/kg or placebo. Loading doses were administered on days 1 and 15, followed by routine treatment every 4 weeks and at a stable dose for ≥3 months.

Results: Among the 198 patients included in the study (ITT population), 62.6% and 37.4% had experienced 2–4 and 5–10 SCPC events in the previous year, respectively, and 62.1% were taking HU at baseline. HbSS was the most common genotype (71.2%, HbSC: 16.2%, HbSPβ0-thalassemia: 6.1%, HbSβ+–thalassemia: 5.1%, other: 1.5%). Overall, more patients in the crizanlizumab 5.0 mg/kg group (n=24/67; 35.8%) were SCPC event-free than in the 2.5 mg/kg group (n=12/66; 18.2%) and placebo (n=11/65; 16.9%) groups. In each of the prior SCPC events, SCD genotype and HU use subgroups, a greater proportion of patients treated with crizanlizumab 5.0 mg/kg were SCPC event-free compared with those in the crizanlizumab 2.5 mg/kg or placebo arms (Table 1). In subpopulations considered to be at increased risk of experiencing a SCPC (patients with 5–10 SCPC events in the previous year and/or with the homozygous HbSS genotype), a higher proportion of patients treated with crizanlizumab 5.0 mg/kg were SCPC event-free compared with those in the placebo arm (28.0% vs 4.2% and 31.9% vs 17.0%, respectively).

Aims: To verify the potential therapeutic use of HpX administration to block NET formation and the occurrence of VOC in human SCD, we aimed to deter...
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 of Hpx were reduced in both VOC and steady state compared to sera from patients with SCD promoted NET formation, which was significantly vented by addition of plasma-derived Hpx. Moreover, exposure of neutrophils to generate reactive oxygen species and release NETs, which was pre-

Summary/Conclusions: In summary, we observed 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).

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) (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).

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Figure 1. OS Kaplan-Meyer Curve (all randomized patients).

New drugs for rescue in relapsed/refractory multiple myeloma

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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.

Figure 1. OS Kaplan-Meyer Curve (all randomized patients).
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OVERALL SURVIVAL OF PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA TREATED WITH CARFILZOMIB AND DEXAMETHASONE VS BORTEZOMIB AND DEXAMETHASONE IN THE RANDOMIZED PHASE 3 ENDEAVOR TRIAL.


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A PHASE IB STUDY OF ISATUXIMAB PLUS POMALIDOMIDE (POM) AND DEXAMETHASONE (DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) (NCT02283775).

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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 5mg/kg (n=8); 10mg/kg (n=12); 20mg/kg (n=6), with Pom 4mg (Days 1–21) and Dex 40mg (Days 1, 8, 15, and 22; 20mg if ≥75 yrs old), in 28-day cycles. An expansion cohort was initiated at ISA 10mg/kg (plus Pom/Dex) based on preliminary safety, efficacy, and PK data. Primary objective: 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 confusion state), and MTD has not been reached. Most common TEAEs, besides IARs, were fatigue (62%), diarrhea (26%), nausea (26%), and constipation (19%). Grade 3/4 hematologic AEs (laboratory assessment) was neutropenia (Gr 3, 40%; Gr 4, 52%). Gr 3/4 thrombocytopenia was reported in 8 (32%) pts (Gr 3, 16%; Gr 4, 16%). IARs occurred in 12 (46%) pts (Gr ≤3 in 1 pt); only with 1st infusion in 9/12 pts. 16 (62%) pts achieved at least PR (5, 8, and 3 pts at 5, 10, and 20mg/kg), including 1 CR, 8 VGPR, 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 of ISA were not affected by co-administration with Pom/Dex.

Summary/Conclusions: The combination of ISA and Pom/Dex was manageable and clinically active in heavily pretreated RRMM. A Phase III trial of this combination is ongoing.

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EFFICACY AND SAFETY OF DARATUMUMAB, BORTEZOMIB AND DEXAMETHASONE (VD) VS BORTEZOMIB AND DEXAMETHASONE (VD) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM) (NCT02283775).

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Background: Daratumumab is a human monoclonal antibody targeting CD38 that induces deep and durable responses with significant clinical benefit and is well tolerated as monotherapy and in combination with established standard-of-care regimens in patients with RRMM.

Methods: Safety data from CASTOR, a multicenter, phase 3, randomized, active-controlled study of Vd versus Vd in RRMM.

Results: Eligible patients with ≥1 prior line of therapy were randomly assigned to 8 cycles (every 3 weeks) of Vd (1.3mg/m2 SC bortezomib on Days 1, 4, 8,
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 3/4 AEs in ≥10% of pts were thrombocytopenia (29%), anemia (15%) and neutropenia (14%). Serious AEs in ≥2 pts were febrile neutropenia, thrombocytopenia, cardiac failure, pyrexia, influenza, lower respiratory tract infection, pneumonia, sepsis, acute kidney injury, respiratory failure, embolism, and hypotension. Dose-limiting toxicities were grade 3 cardiac failure in the 300mg cohort (possibly related to dexamethasone) and grade 3 thrombocytopenia during the first cycle in the safety expansion. No events of laboratory or clinical TLS were reported. Four deaths were due to PD and 1 due to respiratory syncytial virus infection. Overall response rate (ORR) for all pts was 67% (44/66); 28 (42%) pts achieved very good partial response (VGPR) or better (3 stringent complete response [sCR], 10 CR, 15 VGPR). Pts non-refractory to prior proteasome inhibitors (PI) or immunomodulatory drugs (IMiDs) had higher ORR than refractory pts (PI, 92% vs 32%; IMiDs, 82% vs 57%). Among pts refractory to any 2 or more (n=15), 3 or more (n=7), or all 4 (n=4) prior therapies (bortezomib, carfilzomib, lenalidomide, pomalidomide), ORR was 40%, 43%, and 25%, respectively. Median time to progression (~10 vs 3 months) and duration of response (~10 vs 7 months) were longer for pts not refractory to any of these therapies versus refractory pts. ORR for pts with or without cytogenetic abnormalities, respectively, was as follows: 78% vs 65% for t(11;14), 60% vs 67% for t(4;14), 47% vs 73% for del(17p), and 63% vs 69% for del(13q).

Summary/Conclusions: VEN combined with bortezomib and dexamethasone has an acceptable safety profile with promising anti-myeloma activity, and the highest response rates were observed in R/R MM pts who were not refractory to PI or IMiDs. These data support the ongoing phase 3 trial with this regimen in R/R MM.

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 RRMM, with the greatest benefit observed in patients with 1 prior line of therapy.

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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.
Improving prognostication and front-line therapy in chronic lymphocytic leukemia

S461

CYTOGENETIC COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA: DEFINITIONS, ASSOCIATIONS WITH OTHER BIOMARKERS AND CLINICAL IMPACT; A RETROSPECTIVE STUDY ON BEHALF OF ERIC


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Background: Recent evidence suggests that complex karyotype (CK) identified by chromosome banding analysis (CBA) may be a relevant biomarker for treatment decisions in CLL, especially regarding the response to signaling inhibitors. However, many challenges towards routine clinical application of CBA still need to be overcome.

Aims: Repackaging of 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=1846, 52%) or both (n=1355, 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 (79%/12%/9%: MBL and Binet A were grouped together)/IG somatic hypermutation and clinical aberrations, 381/3580 cases (11%) displayed CK, with no difference in the detection rate between different cell stimulation protocols. CK was significantly enriched for U-CLL and reached only 4 deaths at a median follow-up of 5.2 years. When high-CK aberrations) who carried +12,+19 plus other numerical and/or structural abnormalities and displayed extremely indolent clinical course (median OS not yet reached for the low- and intermediate-CK', n=82, 22%) and ≥5 (‘high-CK', n=99, 26%) aberrations. High-CK CK cases were stratified into those with 3 (‘low-CK', n=200, 52%), 4 (‘intermediate-CK', n=82, 22%) and ≥5 structural and/or numerical cytogenetic aberrations are not equivalent, we assessed the relevance of other numerical cut-offs for CK, while also investigated the impact of the type of aberrations (i.e. structural versus numerical). CK cases were stratified into those with 3 (low-CK', n=200, 52%), 4 (intermediate-CK', n=82, 22%) and ≥5 (‘high-CK', n=99, 26%) aberrations. High-CK cases differed significantly (p<0.05) from the other two subgroups, being enriched for U-CLL and TP53 abnormality (TP53abn i.e. del(17p) and/or TP53 mutations); 299/3308 (9%)/TP53 abn. On univariate analysis, CK was not even in univariate analysis (p=0.57).

Summary/Conclusions: CK defined by the presence of ≥3 numerical and/or structural abnormalities should not be axiomedically considered unfavorable in CLL, representing a heterogeneous group with variable clinical behavior. High-CK with ≥5 chromosomal aberrations emerges as prognostically adverse, independently of clinical stage, IG somatic hypermutation and TP53 status. Prospective clinical validation is warranted before finally incorporating high-CK in risk stratification in CLL.

S462

IS CFR THE TREATMENT OF CHOICE FOR IGHV MUTATED CLL WITHOUT POOR FISH CYTOGENETICS?

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Background: Chemoinmunotherapy (CIT) is the standard treatment for young and fit treatment-naive patients with CLL. The median progression-free-survival (PFS) in patients treated with CIT is about 5-6 years and the overall survival (OS) is increased by 5-10% compared to those treated with chemotherapy only. Patients with mutated IGHV genes (M-CLL) and no 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, paricularly FCR.

Aims: The aim of this study was to analyse the outcome of M-CLL patients with no unfavorable cytogenetics CLL according to the type of therapy.

Methods: We analysed 816 CLL patients from Sant Pau Hospital, Barcelona, Spain; Uppsala University Hospital, Sweden and IRCCS San Raffaele Scientific University, Milan, Italy for whom IGHV mutational status was available. Endpoints were OS and TFS.

Table 1.

<table>
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<tr>
<th>Clinic and biological characteristics</th>
<th>OS (years)</th>
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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 66% (CI, 52-38) for U-CLL cases (p<0.05). TFS at 5 and 10 years was 79% (CI, 77-80) and 66% (CI, 66-66) and 28% (CI, 33-23) 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 (136 without poor-prognosis 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] required therapy. Overtreatment was defined as use of purine analogues (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). The...
median duration of response to first therapy was 42 months (range, 33-52) in M-CLL cases vs 24 months (range, 18-30) in U-CLL patients (p<0.001). 282 patients received a second line of therapy: PA-based therapy (n=95), alkylating agents (n=82), anti-CD20 MoAbs with PA or bendamustine (n=33), anti-CD20 MoAbs with alkylating agents (n=16), BCR-signal inhibitors or BCL2 antiprototic agents (n=12), others (n=59), and unknown (n=5). In 481 of 816 patients in whom detailed information on treatment regimens beyond second-line was available, 99 patients received a third-line treatment including PA-based therapy (n=15), alkylating regimens (n=20), anti-CD20 MoAbs with PA or bendamustine (n=15), anti-CD20 MoAbs with alkylating agents (n=8), BCR or BCL2 inhibitors (n=11), others (n=28) and unknown (n=2); 49 patients received four or more lines of therapy. In M-CLL patients without poor FISH cytogenetics (n=136) the type of therapy did not impact patients’ outcome. Thus, the median survival was not reached in patients treated with CIT as first-line (i.e FCR, BR) as compared to 202 months in those not having received CIT (p=0.317). In contrast, in U-CLL patients the OS was highly dependent on the type of therapy. In detail, U-CLL patients who received anti-CD20 MoAbs with PA or bendamustine either as first line or subsequent lines (60 of 120 patients) showed significantly longer survival than those who did not receive these therapeutic regimens (median survival: 173 vs 103 months, p=0.001). On the contrary, in M-CLL cases no differences in survival were observed in those receiving anti-CD20 MoAbs with PA or bendamustine vs who did not (p=0.538).

Summary/Conclusions: This retrospective study suggests that OS of CLL patients with mutated IGHV genes and no unfavorable FISH cytogenetics do not depend on the type of therapy. This has important clinical implications and provides background for randomized studies aimed at identifying the optimal treatment strategy for this group of patients.

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IBRUTINIB, FLUDARABINE, CYCLOPHOSPHAMIDE, AND OBINUTUZUMAB (IAGO101) FOR PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH MUTATED IGHV AND NON-DEL(17P)
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Background: Patients with mutated IGHV (IGHV-M) have favorable long-term outcomes (10-year PFS of >60%) after receiving first-line FCR. Aims: To develop an FC-based chemoinmunotherapy regimen of finite duration that included ibritinib and obinutuzumab. The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through the addition of ibritinib and a more potent antibody (obinutuzumab).
Methods: We designed an investigator-initiated phase II trial with ibritinib, fludarabine, cyclophosphamide, and obinutuzumab (IAGO101 for previously untreated CLL pts). The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through addition of ibritinib 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 ibritinib (ibrutinib) C7-12. Pts not achieving primary endpoint received iG (C4-12). All pts who are MRD neg at 1 year will stop all therapy, including ibritinib. Pts MRD+ at 1 year may continue ibritinib. historic C3 BM MRD-neg with FCR in IGHV-M 26% (Strati, Blood 2014). Target BM MRD-neg after IFCG x3 is 45%. Sample size 45.
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 is 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/µL (range, 3.1-224), platelet count 120 K/µL (range, 62-292), 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 1 month (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 30-70ml/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 ≤5 cm): 9 pts (15%); intermediate (ALC >25.000 µL or LN 5-10 cm): 35 (58%) and high (ALC >25.000 µL & LN 5-10 cm: 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-
ed (table 1); with an ORR of 97%, at the end of induction, the primary endpoint was met. MRD negativity (<10⁻⁸) by flow cytometry in peripheral blood (PB) was achieved in 56 pts (89%); MRD assessment from bone marrow was available in 8 pts (4 TN and 4 R/R, among them 4 with a CR and 4 with a PR) and were all negative. As of January 9th 2017, 83 serious adverse events (SAEs) were reported in 37 pts, including 69 SAEs (83%) related to study treatment. 66 pts (44%) were CRT-3 (0%-80%) and 1 had a fatal adverse event (sepsis-CT04 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 CTC3-5) and hematologic disorders (18 in 10 pts; CTC3-4), followed by infusion-related reactions (6 in 6 pts); laboratory TLS (6 in 5 pts). CRT-1 and CRT-2 were the most incidences during the first 1 in induction cycle 1 with C.G. in cycle 3 and 1 in cycle 4 with G and A) and ischemic coronary artery disorders (5 in 4 pts). No clinical TLS occurred.

Summary/Conclusions: With an ORR of 97% and a MRD negativity of 89%, CRT-1 and CRT-2 were the most incidences during the first 1 in induction cycle 1 with C.G. in cycle 3 and 1 in cycle 4 with G and A) and ischemic coronary artery disorders (5 in 4 pts). No clinical TLS occurred.

CLINICAL AND BIOLOGIC COVARIATES OF OUTCOMES IN ZUMA-1: A PIVOTAL TRIAL OF AXIATIBAGENE CILOUELC (AXI-CELL; KTE-C19) IN PATIENTS WITH REFRACTORY AGGRESSIVE NON-HODGKIN LYMPHOMA (NHL) - RESULTS FROM THE INTERIM ANALYSIS (N=62)

Background: For pts with refractory aggressive NHL are poor with current therapies (Crump, ASCO 2016). Results from the interim analysis of (n=62) pts from the ZUMA-1 trial: Axi-cell, 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 (n=128) diffused large B cell lymphoma (DLBCL), primary mediastinal B cell lymphoma (PMBCL) or transformed follicular lymphoma (TFL); an ECOG performance status (PS) 0-1, refractory disease or 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 axicel. Median age was 58 y (range, 23-76), 67% male, 85% stage III-IV, 47% PS 0-1, 77% refractory to ≥2nd line of therapy, and 21% relapsed ≤12m of ASCT. Axicel 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<0.0001) the study met the primary endpoint. The ORR in the mITT analysis of set 1 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 6.2m 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 (68%), leukopenia (44%), anemia (43%), febrile neutropenia (22%), and encephalopathy (21%). Gr ≥3 cytokine release syndrome (CRS) and neurologic events (NE) occurred in 13% and 28% of pts, respectively. All CRS and 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-cell were associated with durable responses. Additionally, this presentation will include an expanded analysis of efficacy outcomes by novel bio-logic and clinical covariates including key molecular phenotypes and tollizumab/corticosteroid interventions used for management of adverse events.

Summary/Conclusions: Axi-cell significantly improved ORR in patients with refractory aggressive NHL. The CR rate was 7-fold higher compared to historic controls (Crump, ASCO 2016), and nearly all AEs associated with this regimen were manageable. Additionally, these data confirm that axi-cell induced overall survival benefit.”

Drs Locke and Neelapu contributed equally to this study
Background: CC-122 is a cereblon modulating agent that degrades Aiolos and Ikaros, resulting in potent anti-lymphoma and immunomodulatory effects on T- and NK-cell function. Phase 1 clinical data revealed promising activity of CC-122 against follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Preclinical combination of CC-122 with obinutuzumab has shown synergism in FL and additive effects in DLBCL vs obinutuzumab alone in CC-122 preclinical models of patients with relapsed or refractory (R/R) B-cell non-Hodgkin lymphoma (NHL).

Methods: Patients at study entry must have R/R CD20+ B-cell NHL after ≥1 prior regimens for FL/marginal zone lymphoma (MZL) and ≥2 regimens and/or ASCT for DLBCL. CC-122 was given orally (5 of 7) for 28-d cycles in escalating doses plus a fixed dose of intravenous obinutuzumab 1000mg on d2, 8, 15 of cycle (c1) and d1 of c2-8, upon informed consent. CC-122 was continued until progressive disease (PD) or unacceptable toxicity. CC-122 active ingredient in capsule formulation (AIC) 1, 2, 3, and 4mg and CC-122 formulated capsules (F6) 3 and 4mg were evaluated in separate cohorts. Primary endpoints included safety and tolerability, non-tolerated dose (NTD), and maximum tolerated dose (MTD). Response was assessed using the international Cheson 2007 criteria every 2 cycles to c6, every 3 cycles to c12, and every 6 cycles thereafter.

Results: As of January 12, 2017, 34 R/R B-cell NHL patients with DLBCL (n=18), FL (n=15), or MZL (n=1) were enrolled. At study entry, median age was 60 y (26-81), most patients were male (68%), and Ann Arbor was extended stage III/IV in 76% of patients. Of the 18 DLBCL patients, 8 had transformed FL. Of the 16 FL/MZL patients, 44% relapsed in <12 months after first-line treatment. The median number of prior regimens was 4 (range, 1-11), and 13 (38%) patients had received prior SCT. One patient experienced a dose-limiting toxicitiy (DLT) of grade 4 neutropenia (CC-122 dose level of AIC 3mg); no dose was yet an NTD. Median CC-122 duration was 22 wks (range, 3-71) equivalent to 6 cycles (range, 1-18). CC-122 dose reduction or temporary interruption occurred in 10 (29%) or 26 (76%) of patients, respectively, primarily due to adverse events (AEs). Most patients (56%) had <1 wk of interruption due to AEs. The most common (≥10%) grade 3/4 treatment-emergent AEs (TEAEs) were neutropenia (50%) and thrombocytopenia (21%). Fifteen patients (44%) had ≥1 serious TEAE, including 2 each of febrile neutropenia (related to CC-122), cytokine release syndrome (related to obinutuzumab), and pneumonia. Three deaths occurred during the study (2 P1A; 1D2). Overall response rate (ORR) was 59%, including 26% CR and 32% PR (Table 1). Median time to best response was 57 d, and median duration of response was not yet reached. In evaluable patients, 6-mo progression-free survival (PFS) was 63%.

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 ≥100mg every 28d (FL) or 21d (DLBCL) for 6 cycles remain worthy of further study. Updated analysis of CC-122 in R/R B-cell NHL pts. Safety and efficacy data will be updated at the time of presentation.

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POLATUZUMAB Vedotin Plus BENDAMUSTINE And RITUXIMAB Or OBINUTUZUMAB In Relapsed/refractory Follicular lymphoma Or diffuse large b-cell lymphoma: Updated results of a phase 1b/2 study

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Background: Transplant ineligible patients (pts) with relapsed/refractory (R/R) FL or DLBCL have poor outcomes. Polatuzumab vedotin (pola) is an antibody drug conjugate that targets delivery of the microtubule inhibitor MMAE to cells expressing CD79b. Pola + rituximab (R) previously showed promising responses in R/R FL and DLBCL. Adding bendamustine (B) to pola-R improved pola activity in R/R FL and DLBCL patients with relapsed or refractory (R/R) B-cell non-Hodgkin lymphoma (NHL).

Aims: The current phase I/II study (EUDRACT 2014-003333-26; NCT02417285) evaluates the safety and efficacy of CC-122 (4mg capsule) + obinutuzumab (1000mg every 28d) in R/R FL and DLBCL, including R/R FL and DLBCL pts. Safety and efficacy data will be updated at the time of presentation.

Methods: All pts provided informed consent to participate in the study and were treated with pola (1.8mg/kg) + B (90mg/m²) and R (375mg/m²) or G (1000mg) every 28 days (FL) or 21 days (DLBCL) for 6 cycles. Responses were assessed by modified Lugano criteria after 3 cycles, end of treatment (tx), and every 6 months for 2 years following (fu).

Results: As of 14 Nov 2016, 65 pts were enrolled: 24 pts (12 FL, 12 DLBCL) in Pts (n=32) were median age 63 y (37-86), 82% ECOG 0-1 and 6% ECOG 2, 44% FL/PI1P3-5, 78% Stage III/IV, 2 (1-7) median lines of prior tx, 38% refractory to last tx, 13% prior transplant (BMT). DLBCL pts (N=32) were median age 66 (30-86), 88% ECOG 0-1 and 13% ECOG 2. 59% IPI 3-5, 75% Stage III/IV, 2 (1-7) median lines of prior tx, 41% refractory to last tx, 13% prior transplant (BMT). CC-122 doses of ≥3mg and obinutuzumab ≥1000mg every 28d (FL) or 21d (DLBCL) for 6 cycles remain worthy of further study of this combination’s therapeutic potential.

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.

S469

SINGLE AGENT ORAL SELINEXOR EXHIBITS DURABLE RESPONSES IN RELAPSED/REFRACTORY LARGE B-CELL LYMPHOMA (DLBCL) OF BOTH GCB AND NON-GCB SUBTYPES: THE PHASE 2B SADAL STUDY

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In this clinical study we assess the efficacy of single agent SEL in pts with R/R DLBCL. Methods: Pts with R/R DLBCL were randomized to 60 or 100mg of SEL twice weekly (8 doses) per 28-day cycle. Pts were also stratified by DLBCL subtype (GCB or non-GCB). The primary objectives are to determine the ORR and evaluate the safety of 60 vs 100mg doses. Disease response was assessed by an Independent Central Radiological Review (ICRR), using the Lugano Classification (Cheson, 2014). Results: 72 pts were enrolled: 37 pts on 60mg (24 M/ 13 F, median age 71 yrs) and 35 pts on 100mg (23 M/ 12 F, median age 68 yrs). Both groups had a median of 3 prior treatment regimens. The most common related adverse effects (AEs) across both dose groups (Grade 1/2) were: fatigue (46%), nausea (46%), anorexia (42%), and vomiting (33%). Common Grade 3/4 AEs were: effects (AEs) across both dosing groups (Grade 1/2) were: fatigue (47%), nausea (46%), anorexia (42%), and vomiting (33%). Common Grade 3/4 AEs were: thrombocytopenia (39%), fatigue (18%), neutropenia (18%), and anemia (13%). These were managed with dose interruption/reduction, platelet stimulants, and/or standard supportive care. Grade 3/4 fatigue (26% v 11%) and thrombocytopenia (48% v 32%) were higher in 100mg arm as 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, remain 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.

### 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>CR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All doses</td>
<td>63</td>
<td>18 (28.6%)</td>
<td>7 (11.1%)</td>
<td>11 (17.4%)</td>
<td>9 (14.2%)</td>
<td>27 (42.8%)</td>
</tr>
<tr>
<td>60mg</td>
<td>32</td>
<td>10 (31.2%)</td>
<td>6 (18.7%)</td>
<td>4 (12.5%)</td>
<td>5 (15.6%)</td>
<td>12 (37.5%)</td>
</tr>
<tr>
<td>100mg</td>
<td>31</td>
<td>8 (25.8%)</td>
<td>3 (9.7%)</td>
<td>6 (19.3%)</td>
<td>6 (19.3%)</td>
<td>12 (38.7%)</td>
</tr>
<tr>
<td>All patients</td>
<td>31</td>
<td>18 (58.1%)</td>
<td>7 (22.6%)</td>
<td>4 (12.9%)</td>
<td>6 (19.3%)</td>
<td>16 (51.6%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: SEL monotherapy shows activity in pts with R/R DLBCL including in pts with GCB subtype. 60mg SEL twice weekly was more tolerable than 100mg twice weekly, with fewer interruptions due to toxicity. Objective responses to SEL were durable at 60mg BIW, suggesting these responses were associated with clinical benefit.
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

Aims:

Evaluate the maximum tolerated dose (MTD), pharmacokinetic (PK) and pharmacodynamic (PD) profiles, safety, and clinical activity of enasidenib in pts 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 3-4 investigator-reported Tx-related adverse events included indirect hyperbilirubinemia (12%) and IDH-inhibitor-associated differentiation syndrome (IDH-DS; ie, retinoic acid syndrome) (7%). Of 176 R/R AML pts, 94 (53%) had received ≥2 prior AML-directed therapy. Overall response rate (ORR; complete remission [CR] + CR with incomplete count recovery - incomplete remission [CRi] + complete remission with incomplete hematologic recovery - incomplete remission [CRh]) in R/R AML pts was 40.3%, including 34 pts (19.3%) who attained CR (Table). Median time to 1st response was 1.9 months (mos); 87.3% of responding pts attained a 1st response by cycle 5. Median response duration was 5.8 mos. Of pts who achieved CR, 7 pts (21%) did so by cycle 3, 23 (88%) by cycle 5, and 28 (82%) by cycle 7. Median duration of CR was 8.0 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).

SAFETY AND EFFICACY OF VENETOCLAX (VEN) IN COMBINATION WITH DECITABINE OR AZACITIDINE IN TREATMENT-NAIVE, ELDERLY PATIENTS (≥65 YEARS) WITH ACUTE MYELOID LEUKEMIA (AML)

Aims: Newly diagnosed patients (pts) with AML aged ≥65 years and ineligible for standard induction therapy have limited treatment options, and low overall survival. VEN is an orally bioavailable, selective BCL-2 inhibitor that has displayed single-agent activity in pts with relapsed/refractory AML. VEN at escalating doses combined with hypomethylating agents (HMAs) has demonstrated antileukemic activity, with an overall response rate (ORR) including complete remission [CR] and CR with incomplete marrow recovery of 60%. Combining VEN with HMAs, such as decitabine (DEC) or azacitidine (AZA), may provide a novel low-intensity approach for treating AML. Preliminary results from the expansion stage of a phase 1b trial comparing 2 doses of VEN plus either DEC or AZA (NCT02203773) are reported.

Aims: To evaluate the safety and efficacy of VEN at 400-mg vs 800-mg doses plus DEC or AZA.

Methods: This open-label, nonrandomized, two-stage phase 1b study evaluated the safety and efficacy of VEN plus DEC or AZA in treatment-naive pts ≥65 years with newly-diagnosed AML. Eligibility criteria included: ECOG PS ≤2; ineligible for standard induction therapy; intermediate- or poor-risk karyotype. Pts received DEC (Arm D, 20mg/m²/day [d]; intravenous [IV]) on 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-expansion stage consisted of 2 VEN dose cohorts (Arm D, 20mg/m²/d; intravenous [IV]) followed by cycle 7. Median duration of CR was 8.0 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: VEN 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).
Results: As of 13/09/16, 100 pts were enrolled in the expansion stage; 25 pts in each arm. Overall, 61% pts were male; 59% had ECOG PS 1 and 15% ECOG PS 2; mean age was 73.9 (range 65–86); 53% had adverse karyotype; and 22% had secondary AML. Median time on study was 6 (4–9), 6 (0.2–9), 5 (0.5–9), and 4 (1–8) mo for arms D1, D2, E1, and E2, respectively. The incidence of adverse events (AEs) was generally comparable between the 4 arms. Overall, the most common treatment-emergent AEs (TEAEs; ≥30% of pts) were nausea (59%), diarrhea (42%), febrile neutropenia (FN; 41%), constipation (39%), fatigue, and decreased white blood cell count (31% each). The most frequent grade 3/4 TEAE and serious AE was FN (41% and 29%, respectively). No TLS was observed. Overall, 29 pts discontinued the study for ≥1 reason, including progressive disease (PD) per protocol (n=10), “other” (n=10; 9/10 proceeded to stem cell transplantation) and AEs not related to progression (n=10). A total of 16 deaths occurred; 12 pts died within 30 d of initiating VEN and HMA due to AEs (n=12) and PD (n=1). The ORR was 68%, with rates of 76% (19/25), 71% (17/24), 68% (17/25), and 60% (15/25) observed in arms D1, D2, E1, and E2, respectively. The Kaplan-Meier survival curve for all pts with a median follow-up time of 5.4 mo is shown.

Summary/Conclusions: Overall, the safety profile was favorable when combining VEN at either dose with DEC or AZA in treatment-naive elderly AML pts. Promising activity with high ORRs was observed at the lower 400-mg VEN dose in both HMA arms. A Phase 3 study of VEN plus AZA is planned.

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UPDATED SAFETY AND EFFICACY RESULTS OF PHASE 1/2 STUDY OF VENETOCLAX PLUS LOW-DOSE CYTARABINE IN TREATMENT-NAIVE ACUTE MYELOID LEUKEMIA PATIENTS AGED ≥65 YEARS AND UNFIT AML


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Background: Incidence of acute myeloid leukemia (AML) increases with age, and patients (pts) ≥65 years have a poor prognosis, with 5-year survival rates of <10%. Treatment with low-dose cytarabine (LDAC) in this population results in modest complete remission (CR)/CR with incomplete blood count recovery (CRI) rates of 11–19%, including 33% in ≥75 years of age. Venetoclax, an orally bioavailable, potent, selective BCL-2 inhibitor, has shown single-agent activity in heavily pretreated pts with relapsed/refractory AML (Konopleva et al 2016). In combination with LDAC, the recommended phase 2 dose (RP2D) of VEN is 600mg QD (Lin et al, ASCO 2016); preliminary data show the combination is feasible and exhibits significant and durable activity in older AML pts ineligible to receive intensive induction chemotherapy (Wei et al, ASH 2016). Updated safety and efficacy data from the RP2D 600-mg dose cohorts of this study (NCT02287233) are presented.

Aims: Evaluate the safety and efficacy of VEN+LDAC in older pts with untreated AML.

Methods: In this open-label phase 1/2 study, pts ≥65 years with untreated AML, ineligible for standard induction chemotherapy, with an ECOG performance status of 0–2 received oral VEN QD on days (d) 1–28 and subcutaneous LDAC 20mg/m2 QD on d 1–10 of each 28-d cycle. VEN target dose evaluation followed a 3+3 design, ranging from 600–800mg; 18 pts were enrolled and the RP2D was established as 600mg. Safety and efficacy of VEN at RP2D were evaluated in the expansion phase. All pts were hospitalized and received prophylaxis before a dose ramp-up of VEN during cycle 1 to mitigate the risk of tumor lysis syndrome (TLS). Adverse events (AEs) were graded by NCI CTCAE V4.0. Pts enrolled as of May 2016 are included in this analysis; data cutoff was August 2016. All pts provided informed consent.

Results: In total, 61 pts, including 8 from phase 1, were treated at the RP2D of 600mg (median age 74 years; ECOG 1–2 70%; adverse karyotypes 31%; secondary AML 44%; prior hypomethylating agent [HMA] 28%). AEs (all grade; ≥30% of pts) including cytopenias were nausea (72%), hypokalemia (46%), diarrhea (44%), fatigue (43%), and decreased appetite (41%). Grade 3/4 AEs (≥1% pts) were febrile neutropenia (34%), hypokalemia (15%), hypophosphatemia (13%), and hypertension (10%). No pts had clinical TLS; 1 pt had laboratory TLS, which was managed. The 30-d and 60-d mortality rates were 3% and 12%, respectively. The CR/CRi rate was 54% (33/61; 21% CR and 33% CRi). The overall response rate (ORR; CR+CRi+PR+UNF) was 61% (37/61). VEN+LDAC was shown to be active across a wide range of cytogenetic mutations and pt profiles (ORR: 70% in pts <75 years; 52% in secondary AML; 47% in pts with adverse karyotypes; 53% in pts with prior HMA). Among response-evaluable pts, those achieving an objective response have longer survival than pts who do not achieve an objective response (Figure 1).

Figure 1.

Summary/Conclusions: VEN (RP2D 600mg) and LDAC exhibited an acceptable safety profile and durable efficacy in pts aged ≥65 years with untreated AML who are ineligible for or unable to receive intensive induction chemotherapy. ORR highly correlated with overall survival, with better survival observed in responders compared with nonresponders. A planned phase 3 randomized trial has commenced.

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PHASE IB/II STUDY OF NIVOLUMAB IN COMBINATION WITH AZACYTIDINE FOR STAND-IND AML PATIENTS (PTS) WITH RELAPSED ACUTE MYELOID LEUKEMIA (AML)

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Background: Blocking PD-1/PD-L1 pathways enhances anti-leukemia responses in murine AML (Zhang et al, Blood 2009). PD-1 positive CD8 T-cells are increased in bone marrow (BM) of pts with AML (Daver et al. AACR 2016). AZA up-regulates PD-1 and interferon-gamma signaling in AML and the up-regulation of PD-1 has been associated with emergence of resistance to AZA (Yang et al., Leukemia 2013).

Aims: To assess the best response to Aza+Nivo at the end of 3 courses of combination therapy.

Methods: Pts were eligible if they had AML and failed prior therapy, had adequate performance status (ECOG ≤2), and organ function. The first six pts...
received AZA 75mg/m² Days 1-7 with nivolumab 3mg/kg on Day 1 and 14. Courses were repeated every 4-5 weeks indefinitely. Only one of six pts had a dose limiting toxicity (grade 3 pneumonitis) and this dose was RP2D. 60 additional pts have been treated at the RP2D.

Results: 66 pts with a median age of 71 years (range, 44-90), secondary AML (39%), poor risk cytogenetics (35%), median number of prior regimens 2 (range, 1-7) have been enrolled. All 66 pts had baseline next generation sequencing: TP53 (n=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); 12 (18%) had stable disease >6 months, and 24 (38%) had progression. 3 pts are too early for response assessment (<3 courses). The median number of courses to CR/CRi/HI was 2 (range, 1-4+). The med OS among the CR/CRi pts was 15.3 months (range, 2.9-27.6). 10 pts was 5.7 months (range, 4.67-17.45+), and NRM 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 med OS with AZA-based salvage protocols in similar pts treated at MDACC (P=0.10) (Fig 1A and Fig 1B). Grade 3/4 and Grade 2 immune toxicities were observed in 8 (12%) and 7 (11%) pts, respectively. The most common Grade 3/4 AEs on treatment included pneumonitis, colitis, nephritis, skin rash, and hypophosphatia. 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-reg cells (P=0.01) infilitrate 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-effector cells at EOC 1 and EOC 2 as compared to those who had no response. The CTLA4 on CD8 T-cells went up in both responders and non-responders after PD1 based therapy.

Summary/Conclusions: Full dose AZA and nivolumab are tolerable and produce an encouraging response rate with durable responses in relapsed AML with poor risk features. Immune mediated toxicities occur and may be adequately managed with early recognition and systemic steroids. Up-regulation of CTLA4 may be a mechanism of resistance to PD1 based therapies in AML and suggest role for combination therapy.

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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 reached mOS of only 1.5 months. (Giles F, et al. Cancer 104 (3), 2005). Such poor-risk pts may benefit from a stem cell transplant (SCT), if available. Aims: The primary aim was to compare SCT rates and outcomes of pts on quizartinib from an exploratory selected cohort in the AC220-002 study with those from a historical cohort of 1388 AML pts with confirmed FLT3-ITD mutations in the UK NCRI database.

Methods: Within AC220-002, 58 pts with a FLT3-ITD mutation were identified who had received intensive chemotherapy, and were relapsed (n=53), or refractory (n=5) to salvage therapy prior to entry. Applying the same entry criteria to the NCRI database, we identified 118 pts who received only recognized chemotherapy regimens prior to eligibility (relapsed n=99; refractory n=19). To avoid biases where those dying early would predominantly contribute to the NCRI group (reflecting that pts in AC220-002 had to be fit enough to be enrolled), pts in this cohort entered analysis 14 days following being identified as R/R. Multivariable Cox/logistic regression was used to compare remission rates and survival stratified for known prognostic factors. A landmark analysis excluding deaths before day 90 (allowing for those too unfit for SCT) was performed on the pooled sample (n=176) of the AC220-002 and NCRI cohorts to compare survival between transplanted and non-transplanted pts.

Results: Overall, quizartinib-treated pts had significantly greater remission rates, consisting mainly of complete remission without normal blood counts (CRi), vs NCRI pts (40% vs 3%, adjusted OR 0.05 (0.01-0.21), p<0.0001) and improved mOS (140d vs 54d, adjusted HR 0.38 (0.25-0.58) p<0.0001). A greater proportion of pts in AC220-002 proceeded to SCT: 23/58 (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 obviously influence treatment choices and outcomes, pts with AML with FLT3-ITD mutation appeared to benefit with longer survival observed with SCT. This data suggests quizartinib may show promise in potentially improving long-term survival by bridging patients to SCT.
Immunotherapy in ALL

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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: The CD19-targeted chimeric antigen receptor (CAR) T-cell therapy CTL019, an investigational therapy that reprograms cytotoxic T cells to eliminate target cells, resulted in high response rates and a manageable safety profile in pediatric/youth adult patients (pts) with R/R B-cell ALL in a single-center trial. Aims: We report an updated interim analysis from the first multicenter global pivotal phase 2/3 study of CTL019 in pediatric/youth adult pts with CD19+ R/R B-cell ALL with ≥5% bone marrow lymphoblasts by morphology. CTL019 was manufactured from leukapheresis products of autologous peripheral blood T cells at a centralized manufacturing facility. The primary endpoint was overall remission rate (complete remission [CR] + CR with incomplete blood count recovery [CRi]) within 3 mo. Secondary endpoints included duration of remission (DOR), overall survival, safety, and cellular kinetics.

Results: As of November 2016, 88 pts were enrolled. There were 7 (8%) manufacturing failures, 9 (10%) pts were not infused due to death or adverse events (AEs), and 4 pts (5%) were pending infusion at the time of data cutoff. Following lymphodepleting chemotherapy in most pts (fludarabine/cyclophosphamide [n=64] or other [n=1]), 68 pts were infused with a single dose of CTL019 (median dose, 3.0×10^6/kg; range, 0.25-6.4×10^6/kg) or placebo (ELIANA; NCT02435849 and ENSIGN; NCT02228096) in pediatric and young adult R/R B-cell ALL were pooled to evaluate cellular kinetics of CTL019. 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.

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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/exttrinsic 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; AUC-28d, 104% higher geo mean. Table 1). Pts with NR had delayed Tmax compared with pts with CR/CRi (20 vs 10 days). Intrinsic pt factors including baseline cytogenetics, disease characteristics, and disease status did not appear to affect Cmax or AUC-28d with the exception that pts with a higher tumor burden at enrollment generally had higher expansion, based on box plots of summary statistics. Extrinsic factors (prior lines of therapy, stem cell transplant) and parameters related to the manufactured product (%) T cells, transduction efficiency, cell viability, total cell count), did not appear to impact cellular kinetics, based on graphical analysis. AUC-28d increased with pres...
ence and severity of CRS. Pts who received anti-cytokine agents for grade 3/4 CRS also had higher expansion. CR/CRI pts treated with tocilizumab and steroids (n=17) had 89% higher AUCO-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 transgenic 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 AUCO-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 AUCO-28d was variable. The boosted levels of anti-CAR19 did not impact clinical response or relapse.

**Table 1.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC0-28d (µg/mL)</th>
<th>y=0.995 (R2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S1</strong></td>
<td>5.1 (3.2, 7.1)</td>
<td></td>
</tr>
<tr>
<td><strong>S2+</strong></td>
<td>6.3 (5.2, 7.1)</td>
<td></td>
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</tbody>
</table>

**Summary/Conclusions:** There was increased expansion of CTL019 in pts with higher tumor burden at enrollment, which correlated with higher CRS grade. There was no relationship between dose and expansion, supporting the wide dose range used. Expansion was not attenuated by tocilizumab 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 (CT). Second and subsequent 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+T-positive cells and CD19-positive B cells to induce tumor cell lysis. In a randomized phase 2 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/Cri) in both the S1 and S2+ groups was higher in the blinatumomab arm compared with the SOC arm (Table 1). Patients randomized to blinatumomab had a median (95% CI) of 11.1 (8.2, NR) months vs 5.1 (3.2, 7.1) months overall survival for S1 vs S2+ subgroup, compared with 5.5 (3.7, 9.0) months vs 3.0 (2.1, 4.0) months in the SOC arm (Figure 1). For both S1 and S2+ subgroups, blinatumomab patients had longer median survival time. Grade 3 or worse AEs were experienced by 61% and 83% of S1 patients in the blinatumomab and SOC group, respectively. These percentages were 68% and 75%, respectively, in S2+ patients. Grade 4 or worse AEs occurred in 34% and 51% S1 patients, and in 36% and 54% S2+ patients. Neurologic events of grade ≥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.**

<table>
<thead>
<tr>
<th>Event</th>
<th>S1 (n=134)</th>
<th>S2+ (n=271)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall Survival</strong></td>
<td>7.7 months</td>
<td>4.0 months</td>
</tr>
<tr>
<td><strong>Grade 3 or worse AEs</strong></td>
<td>61%</td>
<td>83%</td>
</tr>
<tr>
<td><strong>Grade 4 or worse AEs</strong></td>
<td>34%</td>
<td>51%</td>
</tr>
<tr>
<td><strong>Neurologic events of grade ≥3</strong></td>
<td>9%</td>
<td>9%</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** The OS and remission rates in S1 patients were higher with blinatumomab vs SOC. Improved OS compared with SOC regardless of prior salvage therapy. Improved OS compared with SOC in S1 patients supports earlier use of blinatumomab.

**S479**

**DURABLE LONG-TERM SURVIVAL OF ADULT PATIENTS WITH B-ALL AFTER CD19 CAR (19-28Z) T CELL THERAPY**

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**Background:** CD19-specific chimeric antigen receptor (CAR) T cells have demonstrated high initial responses in patients with relapsed B-ALL. However, clinical characteristics associated with the durability of response remain undefined.

**Aims:** We performed a retrospective analysis of our phase I clinical trial of 19-28z CAR T cells in adult patients with relapsed B-ALL (NCT01044069) with a focus to identify those patients who optimally benefit from 19-28z CAR T cell therapy with durable long-term survival and reduced toxicities.

**Methods:** Adults with relapsed B-ALL were infused with autologous T cells expressing the 19-28z CAR following conditioning chemotherapy. Disease burden was assessed by bone marrow biopsy immediately prior to T cell infusion; patients with <5% blasts were classified as minimal residual disease (MRD) cohort vs patients ≥5% blasts as morphologic disease cohort. Response assessment occurred at 4 weeks. Median follow-up duration was 18 months (range, 0.2-57.3).

**Results:** 51 adults received 19-28z CAR T cells; 20 in the MRD and 31 in the therapy with durable long-term survival and reduced toxicities. Complete remission (CR) rates were comparable (95% and 77%, respectively). However, median event-free and overall survivals widely diverged among the 42 patients who achieved MRD-negative CR: not reached (NR) (95% confidence interval [CI]: 4.2-79) 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 disease cohorts, respectively. Subsequent allogeneic HSCT in either cohort did not improve survival (p=0.8). MRD cohort patients developed substantially less severe cytokine release syndrome (CRS) and neurotoxicity, and both toxicities significantly correlated with peak

**Figure 1.**

**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.
CART cell expansion (n=0.0326 and p=0.0001, respectively). No case of cerebral edema was observed.

Summary/Conclusions: Despite comparable initial CR rates regardless of pre-treatment disease burden, duration of 19-28 CART cell mediated remissions and survival in adult patients with relapsed B-ALL positively correlated to a low disease burden and do not appear to be enhanced by allogeneic re-transplant. Our findings strongly support the early incorporation of CD19 CART therapy before morphologic relapse in B-ALL.

S480

STANDARD-RISK RANDOMIZATION OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA TRIAL AIEOP-BFM ALL 2000 INDICATES EQUALLY GOOD OUTCOME WITH REDUCED-INTENSITY DELAYED INTENSIFICATION IN ETV6-RUNX1-POSITIVE PATIENTS

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Background: ETV6-RUNX1 fusion is a common genetic aberration in childhood acute lymphoblastic leukemia (ALL) and is associated with good prognosis in the context of contemporary treatment regimens. The required treatment intensity for this well-described biologic subgroup with low risk of relapse is not known so far. In trial AIEOP-BFM ALL 2000, feasibility of reduced of delayed intensification treatment to reduce the burden of chemotherapy was tested in a randomized approach in the standard-risk group. Treatment reduction was not successful in the total cohort (8-year probability of disease-free survival (8y-pDFS, ± standard error) 89.2±1.3% for reduced delayed intensification treatment, 92.3±1.2% for the standard treatment (log-rank P=0.04) due to evidence of more relapses observed in patients treated less intensively.

Aims: The retrospective subgroup analysis presented here focuses on the ETV6-RUNX1-positive patients included in the group of randomized standard-risk patients.

Methods: From 07/2000 to 06/2006, 4741 eligible patients with ALL (age range 1-17 years) were enrolled in the trial AIEOP-BFM ALL 2000 (NCT 00430118 (BFM) and NCT 00613457 (AIEOP)). Of those, 1164 patients were considered at standard risk of relapse, defined by lack of genetic high-risk criteria and absence of minimal residual disease at day 33 and week 12 of treatment (tested by immunoglobulin/β-cell receptor gene rearrangement polymerase chain reaction). They were randomly assigned to either receive the reduced-intensity protocol (P-II) or the standard protocol (P-I) for delayed intensification. P-III is shorter than P-II (duration 29 vs 49 days), the dose of dexamethasone in P-III is 30% lower, and the dose of vincristine, doxorubicin, and cyclophosphamide are reduced by 50% as compared to P-II. The intention was to prove non-inferiority of the reduced-intensity treatment compared to standard treatment.

Results: ETV6-RUNX1-positive patients (n=367) accounted for 34% of randomized standard-risk patients (Age: <8 years n=260, 8 to <10 years n=79, ≥10 years n=28; early cytologic response evaluation in bone marrow on day 15 of induction treatment: M1 n=218, M2 n=74). Of those, 188 were treated with the experimental P-III, 179 received the standard P-II. With a median follow-up of 8.6 years, the as-treated analysis showed an 8y-pDFS of 94.5±1.7% for P-II and 94.4±1.8% for P-III (log-rank P=0.74). Cumulative incidence of relapse at 8 years was 3.3±1.3% and 4.3±1.6% (Gray P=0.09), and 8-year overall survival was 96.9±1.4% and 98.8±0.9% (P=0.27) for P-III and P-II, respectively. Analysis of ETV6-RUNX1-positive patients by age groups or treatment response on day 15 allowed no further refinement of prognostic subgroups.

Summary/Conclusions: There was no evidence of prognostic disadvantage in ETV6-RUNX1-positive standard-risk patients when treated with the reduced-intensity experimental arm. No clear age- or response-dependent differences could be revealed for this group, which is in line with the biologic understanding of this genetic subgroup. Hence, it might be postulated that treatment reduction might be possible in this well-defined biologically defined subgroup. However, the current data is not sufficient to indicate non-inferiority study question focused on the subgroup of ETV6-RUNX1-positive patients, but reflects a subgroup analysis with descriptive character. Therefore, any decision for treatment reduction should be considered carefully.

S481

A SECOND GENERATION LYOSOMOTROPIC AGENT DRIVES LEUKAEMIC STEM CELL DIFFERENTIATION AND SENSITIZES THEM TO TYROSINE KINASE INHIBITOR TREATMENT IN VITRO AND IN VIVO

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1Wolfson Wohl Cancer Research Centre, Institute of Cancer Science, University of Glasgow, 2Cancer Research UK, Beatson Institute, Glasgow, United Kingdom, 3Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States, 4Paul O’Gorman Leukaemia Research Centre, Institute of Cancer Science, University of Glasgow, Glasgow, United Kingdom

Background: Autophagy is a conserved catabolic process that delivers cytoplasmic constituents to the lysosomes. We have previously shown that the lysosomotropic agent hydroxychloroquine (HCQ) inhibits autophagy and sensitizes Chronic Myeloid Leukaemia (CML) stem cells (LSCs) to tyrosine kinase inhibitors (TKIs) treatment. However, the biological effects of autophagy inhibition in LSCs in vivo are currently unknown and remain to be investigated. Furthermore, recent clinical studies showed that maximum tolerated dose of HCQ does not achieve consistent autophagy inhibition in cancer patients. Therefore further pre-clinical studies using more potent 2nd generation lysosomotropic agents, alone and in combination with TKIs, are vital.

Aims: Here we aim to investigate the functional effects of autophagy inhibition in LSCs both in vitro and in vivo using the highly potent lysosomotropic agent Lysosome 05 (Lys05). Additionally, we aim to address whether Lys05 achieves autophagy inhibition in the most primitive LSC populations in vivo and whether it targets LSCs more effectively than HCQ when combined with TKIs.

Methods: In this study, we used primary stem-cell enriched samples (CD34+ cells) derived from CML patients at diagnosis. For in vivo studies, we used a human patient-derived xenograft (PDX) model and an inducible transgenic CML model in which the expression of BCR-ABL is induced at a stem/progenitor level (Scl-Ta-BCR-ABL). To accurately measure autophagy flow in long term LSCs in vivo, we generated the transgenic mouse Scl-Ta-BCR-ABL/GFP-LC3 by crossing the Scl-Ta-BCR-ABL mouse with a mouse bearing the autophagy reporter 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 CFSEFC594/CD34+CD133+ cells respectively. Interestingly, Lys05 promoted a 40% decrease of quiescent cells and induced myeloid differentiation of CD34+ cells. Functional long-term culture initiating cell (LTG-1) 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-Ta-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+CD48-CD150+ followed by a 50% increase (p=0.0231) of progenitors Lin+Sca+c-kit-. This result indicates differentiation of LSCs towards a more progenitor phenotype following potent autophagy inhibition. Finally, to test the in vivo effect of Lys05, we transplanted CML cells into irradiated NSG mice. Remarkably, using this PDX model we show that while 3 weeks in vivo treatment with HCQ had no effects when combined with TKIs, Lys05 and TKI treatment nearly eliminated engrafted primitive Philadelphia positive CD34+CD38+ and CD34+CD133+ cells.

Summary/Conclusions: Overall, we demonstrate that lysosomal inhibition induces loss of quiescence and drives differentiation of LSCs in vitro and in vivo. Furthermore, our results show that Lys05 achieves autophagy inhibition in LSCs and effectively sensitizes LSCs to TKIs in vitro and in vivo. Therefore, 2nd generation lysosomotropic agents should be considered as a potential alternative to HCQ in order to eliminate LSCs and achieve cure for CML patients.

S482

FC GAMMA RECEPTOR 2B IS CRITICAL FOR BCR-ABL MEDIATED LEUKEMOGENESIS

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Background: Chronic myeloid leukemia (CML) is provoked by the chromoso-
S483 MYC-DEPENDENT REPRESSION MECHANISM OF THE MI-R150 TRANSCRIPTIONAL REGULATION IN CHRONIC MYELOID LEUKEMIA P. Burda1,2, N. Čuřík1,2,*, K. Šrůtová1, F. Savvulidi2, G. Silvestri3, H. Klamová1,4, P. Pecherková1, Ž. Sovová1, J. Koblihová1, T. Stopka5, D. Perrotti3, K. Machová1, I.S. Pagani1, P. Dang1, I.O. Kommers2, J. Goyne1, V.A. Saunders1, J.A. Prime3, D.L. White1, S. Branford3, T.P. Hughes1, D.M. Ross4,* 1Cancer Theme, South Australian Health and Medical Research Institute (sahrni), Adelaide, Australia, 2School of Medicine, Vrije Universiteit Medical Center, Amsterdam, Netherlands, 3Department of Genetics and Molecular Pathology, Centre for Cancer Biology,SA Pathology, 4Department of Haematology, Royal Adelaide Hospital and SA Pathology, Adelaide, Australia

Background: Real-time reverse transcription quantitative PCR (RQ-PCR) for BCR-ABL1 mRNA is widely used for the monitoring of chronic myeloid leukaemia (CML). Pre-analytical factors, such as the rate of degradation of the target mRNA, and methodological factors, such as the choice of control gene, may influence the final result. In contrast the genomic DNA is stable, and the number of copies of BCR-ABL1 DNA is directly proportional to the number of CML cells. Measuring both DNA and RNA may enable us to understand the contribution of expression and cell number to the RQ-PCR response.

Aims: To compare BCR-ABL1 DNA Q-PCR and routine RQ-PCR monitoring of CML.

Methods: Fifty-nine newly diagnosed chronic phase CML patients from the ALLG CML9 (TIDEL II) trial were included in this sub-study. Samples were tested prior to commencing TKI treatment (baseline), at 1, 2, and 3 months, and every 3 months to 24 months (total 568 samples). Since we wanted to compare the expressivity of the Q-PCR methods we selected patients who had achieved undetectable minimal residual disease (UMRD) by RQ-PCR within 24 months, and an additional 40 patients unsellected for response. RQ-PCR results were expressed on the International Scale (IS), whereas DNA results were expressed relative to the individual patient’s baseline. Quantification of BCR-ABL1 DNA by digital PCR (dPCR) using the Fluidigm BioMark HD System. The mean detection limit of RQ-PCR was 4.5-log, and 5.4-log for DNA methods.

Results: We first demonstrated that DNA dPCR and real-time Q-PCR gave comparable results: 45 samples from 6 patients were quantified by both methods and logarithmically transformed. The mean bias was -0.15 (1.4-fold) with comparable results: 45 samples from 6 patients were quantified by both methods and logarithmically transformed. The mean bias was -0.15 (1.4-fold) with 95% limits of agreement ranging from -1.19 to 0.88. Subsequently, DNA and mRNA values were compared in paired samples. The median BCR-ABL1 mRNA at baseline was 58% (range, 2.4% - 487%) versus 93% by DNA methods (range, 2.4% - 235%). Interestingly, BCR-ABL1 DNA was significantly higher than mRNA at 1, 2, and 3 months (Figure). There was good agreement between positive results from 6 months of TKI therapy onwards (mean bias -0.02: 95% limits of agreement from -1.15 to 1.11). Comparing the limit of detection, BCR-ABL1 DNA was detectable in 60/148 (41%) samples with undetectable mRNA.
Aims: The first phase of the study was aimed to i) create a network of 4 labs conducting to assess the feasibility, cost, turnaround times and clinical utility of a next generation amplicon deep sequencing (Deep Seq) strategy for routine kinase domain (KD) mutation screening is a precious tool for timely and rational benefit from sensitive BCR-ABL1 KD mutation screening by Deep Seq. In the second phase, pts positive for mutations were 25/159 (16%; 23 Failures and 2 Warnings) by Deep 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. Compound mutations found only in 2 out of 52 mutated pts (both in blastic phase). Summary/Conclusions: 1) Results of the ‘NEXT-IN-CML’, the first prospective study evaluating the routine diagnostic use of Deep Seq of BCR-ABL1, show that this technology can successfully be implemented in national lab networks and is feasible, robust and reproducible; 2) in a relatively large, nonselected cohort of CML pts analyzed for mutations because of a Failure or Warning response, Deep Seq confirmed that enhancing sensitivity enables to detect BCR-ABL1 KD mutations in twice as many pts as compared to Sanger Seq (33% vs 16%); 3) all the pts who need to be switched to another TKI would benefit from sensitive BCR-ABL1 KD mutation screening by Deep Seq. 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.

**S485**

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

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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 conducting 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.

**Methods:** In the first phase, centrally prepared identical batches of 32 blinded samples (24 clinical samples with known mutation status/load as assessed by Sanger Seq plus 8 T315I+ BaF3 cell line dilutions simulating mutation loads between 20% and 1%) were distributed and analyzed in parallel by each of the 4 participating labs. In the second phase, 159 consecutive CML pts were prospectively studied in parallel by Sanger Seq and by Deep Seq: 101 Failures (57 pts on 1st-line TKI [IM, n=38; DAS, n=12; NIL, n=7] therapy; 35 pts on 2nd-line TKI [DAS, n=14; NIL, n=17; IM, n=2; BOS, n=1; PON, n=1] therapy; 5 pts on 3rd-line TKI [DAS, n=4; NIL, n=1] therapy and 4 pts on 4th-line TKI [BOS, n=1; NIL, n=4; DAS, n=4; NIL, n=5; BOS, n=1] therapy and 20 on 2nd-line TKI [NIL, n=10; DAS, n=9; PON, n=1] therapy).

**Results:** In the first phase, 504/512 amplicons were successfully generated and sequenced, with a median number of forward and reverse 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 Deep 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 were found only in 2 out of 52 mutated pts (both in blastic phase).
Prognostic markers and new treatment in MDS

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

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Background: Cytopenia is a hallmark in myelodysplastic syndrome (MDS), however, many patients with persistent cytopenia do not fulfill the criteria for MDS. These patients are now classified as idiopathic cytopenia of undetermined significance (ICUS) or if a mutation is detected as clonal cytopenia of undetermined significance (CCUS). Little is known about these new entities in regards to survival and prognostication.

Aims: In this study we want to compare ICUS patients with MDS patients having low- or very low-risk disease according to the IPSS-R. We also wanted to investigate if sequencing of the cohort could bring additional information in regards to overall survival.

Methods: All patients underwent a bone marrow biopsy, cytogenetics and a broad range of blood tests. Furthermore, all ICUS patients underwent a blinded morphology review by two experienced pathologists; these review data will be ready for presentation at EHA. ICUS was defined as persistent cytopenia for more than six months, no chromosomal aberrations and common causes of cytopenia were ruled out. The patients were sequenced with a targeted sequencing panel, either using a customized Haloplex panel or a customized sequencing panel for the Ion Torrent platform. We analyzed 20 genes which are the most commonly mutated genes in MDS.

Results: So far we included 157 patients, 122 were classified as ICUS and 35 as MDS and the median age is 65 and 68 years, respectively (p=0.27). We have sequenced 78% of the ICUS patients and 74% of the MDS patients. In total 53% and 73% of the ICUS and MDS patients had at least one mutation detected, respectively. If the patients carried a mutation, the median number of mutations was two in both the CCUS and the MDS group. The most commonly mutated genes were TET2, SRSF2, DNMT3A and ASXL1 in 38 patients (31%), n=16 (13%), n=10 (8%), n=10 (8%), respectively. There were no significant differences in the distribution between the two groups. Mutations in NRAS, KRAS, TP53 were only identified in one patient each. The overall survival between the ICUS and the low-risk MDS patients did not differ (p=0.18) (figure 1). We also subdivided the ICUS patients into non-clonal ICUS and CCUS, but observed no difference between these two groups (p=0.355).

Eight of the patients categorized as ICUS progressed to a myeloid neoplasm during the follow up, and of these seven had a detectable mutation at time of progression, no differences in response were observed based on PD-1 expression. Patients with previously untreated MDS were 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 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 treated to be in one of 3 consecutive cohorts combining AZA 75mg/m2 iv daily days 1-5 of a 28 day cycle with: cohort #4: Nivo 3mg/kg iv Days 6 and 20; cohort #5: Ipi 3mg/kg iv on day 6; and cohort #6: Nivo 3mg/kg iv on days 6 and 20 + Ipi 3mg/kg iv on day 6. The maximum size per cohort is 20 pts. The primary endpoint is to determine the safety of Nivo or Ipi as single agents or in combination with AZA. Secondary objectives included overall response rate (ORR) and assessment of biological activity. Responses were evaluated following the revised 2006 IWG criteria. The study included stopping rules for response and toxicity.

Results: A total of 63 pts have been enrolled, 54 (86%) are evaluable for response and toxicity including 21 treated with frontline AZA+Nivo, and 15 and 18 with Nivo or Ipi after HMAMA failure, respectively Median age is 69 years (range 39-85). The median number of treatment cycles was 3 (range 1-11). A total of 3 (27%) pts in the AZA+Nivo cohort, 6 (40%) in the Nivo cohort, and 3 (33%) in the Ipi cohort had a relapse or progression. 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.

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AN UPDATE OF A PHASE II STUDY OF NIVOLUMAB (NIVO) OR IPILOMUBAB (IPI) WITH AZACITIDINE IN PTS WITH PREVIOUSLY TREATED OR UNTREATED MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Outcomes of pts with MDS after hypomethylating agent (HMA) failure remain poor. Upregulation of PD-1, PD-L1 and CTLA-4 in MDS CD34+ cells after exposure and loss of response to HMA have been reported. Nivo and Ipi are monoclonal antibody directed against PD-1 and CTLA-4, respectively, with clinical activity in solid tumors.

Aims: To evaluate the potential activity of immune checkpoint antibodies in patients with previously treated or untreated MDS.

Methods: We designed a phase II study of Nivo and Ipi in monotherapy or combination for pts with MDS. Pts with prior therapy with HMA were to be treated in one of 3 consecutive cohorts: cohort #1: Nivo 3mg/kg iv days 1 and 15 of a 28 day cycle; cohort #2: Ipi 3mg/kg iv on day 1 of a 21 day cycle; cohort #3: Nivo 3mg/kg iv on days 1 and 15 + Ipi 3mg/kg iv on day 1 of a 28 day cycle. The study design allowed for AZA add-back after 6 cycles of therapy if there was no response or progression. Pts with previously untreated MDS were to be treated in one of 3 consecutive cohorts combining AZA 75mg/m2 iv daily days 1-5 of a 28 day cycle with: cohort #4: Nivo 3mg/kg iv Days 6 and 20; cohort #5: Ipi 3mg/kg iv on day 6; and cohort #6: Nivo 3mg/kg iv on days 6 and 20 + Ipi 3mg/kg iv on day 6. The maximum size per cohort is 20 pts. The primary endpoint is to determine the safety of Nivo or Ipi as single agents or in combination with AZA. Secondary objectives included overall response rate (ORR) and assessment of biological activity. Responses were evaluated following the revised 2006 IWG criteria. The study included stopping rules for response and toxicity.

Results: A total of 63 pts have been enrolled, 54 (86%) are evaluable for response and toxicity including 21 treated with frontline AZA+Nivo, and 15 and 18 with Nivo or Ipi after HMA failure, respectively Median age is 69 years (range 39-85). The median number of treatment cycles was 3 (range 1-11). A total of 3 (27%) pts in the AZA+Nivo cohort, 6 (40%) in the Nivo cohort, and 3 (33%) in the Ipi cohort had a relapse or progression. No differences in response were observed based on PD-1 bone marrow expression.

Summary/Conclusions: We here demonstrate that low-risk MDS and ICUS patients share similar survival patterns, however, larger studies with longer follow up are needed. Mutations are most commonly found in the epigenetic regulators in this cohort of ICUS and low-risk MDS, while mutations in classical tumor suppressors and oncogenes such as TP53 and NRAS are rare. Mutational screening seems promising in detecting patients at risk of progression, however, other biomarkers for prognostication are warranted.

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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: Azacitidine (AZA) is first line therapy for patients (pts) with higher risk MDS and demonstrated efficacy in older pts with AML (Dombret et al, Blood
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 for clonal heterogeneity testing, 38 (55%) where clonally heterogeneous and carried at least 1 subclone. Pairwise associations of mutations revealed distinct and significant co-mutation patterns (Figure 1B). Within these co-mutation associations, there were no clear hierarchical patterns of clonality in patients evaluable for clonal heterogeneity, as indicated in Figure 1B. By univariate analysis, presence of mutations in ASXL1 (OR 0.45, CI 0.22-0.93, p=0.03) and RUNX1 (0.44, CI 0.20-0.96, p=0.038) as well as that of TP53 mutations with VAF ≥0.31 (OR 0.21, CI 0.05-0.8, p=0.024) predicted for a lower likelihood of response. Analysis of functional pathways revealed that patients with mutations in chromatin (OR 0.43, CI 0.21-0.86, p=0.017) and signaling genes (OR 0.48, CI 0.23-1.00, p=0.049) had lower likelihood of achieving response. Additionally, patients with ASXL1 mutations (OR 0.24, CI 0.09-0.64, p=0.005), particularly in the absence of co-occurring TET2, as well as those with increased number of mutations, particularly if more than 3 (OR 0.21, CI 0.06-0.73, p=0.014), or signaling gene mutations (OR 0.32, CI 0.13-0.80, p=0.016), had a lower likelihood of achieving a CR. Among patients who achieved CR, presence of 3 or more mutations (2.6 vs 1.3 months, OR 1.35, CI 1.00-1.83, p=0.049) and TP53 mutations with VAF ≥0.31 (0 vs 3.7 months, OR 2.03, CI 1.03-3.98, p=0.040) predicted for shorter CR duration. Presence of clonal heterogeneity, as well as the identified pairwise co-mutation patterns did not predict for any of the response outcomes.

Summary/Conclusions: The combination of oral RIG and standard-dose AZA was well tolerated in repetitive cycles in pts with AML and MDS. Response was observed both in HMA-treatment-naive pts (85%) and in pts failing HMA therapy (62%), suggesting the addition of RIG can overcome HMA clinical resistance by acting as a chromatin modifying agent. In AML, responses were seen in 37.5% of evaluable pts. Based on these results, continued study in AML is warranted. A Phase III study of the combination of oral RIG and AZA in pts with treatment naive MDS is planned.

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IMPACT OF THE MUTATIONAL PROFILE AT THE TIME OF DIAGNOSIS IN RESPONSE OUTCOMES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND CHRONIC MYELOMONOCYTIC LEUKEMIA TREATED WITH HYPMETHYLATING AGENTS

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Summary/Conclusions: The type, number and burden of mutations at the time of diagnosis may predict response to therapy with HMA in patients with MDS and CMML.
STUDY OF THE EFFECT OF miRNAs TARGETING RPS14 ON CELLULAR BIOLOGICAL BEHAVIOR OF MYELODYSPLASTIC SYNDROMES

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Background: As key factors in gene post-transcriptional regulation, micro-RNAs (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 specific miRNA could target RPS14 by assay of luciferase activity. Secondly, the expression level of miR223 was measured by qRT-PCR in cell lines and patients. Thirdly, constructing lentivirus which carried miR223 overexpression vector and RPS14 expression was detected by means of immunofluorescence (IF).

Results: 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 PTPN11 (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 (0.9%), 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%).

Summary/Conclusions: MDS patients had higher miR-223 expression compared with health controls. We demonstrated that miR223 could promote cell proliferation, inhibit cell apoptosis and suppress terminal erythropoiesis through target RPS14.
serial samples, we inferred the clonal relationships between original and relapsing disease in 20 patients (Fig B). Mutations from initial diagnosis reappeared in 17 patients. The relapse clone of 13 patients was identical to or clonally evolved from the initial AML clone (7 and 6 patients, respectively). Relapse clones of 4 patients evolved from an inferred ancestral clone, distinct from the initial AML clone. The remaining 3 patients’ relapse clones appear to be new clonal events distinct from any observed in the initial AML clone from relapsed patients, 37 were stable, whereas 9 were cleared and 5 acquired (or selected) at relapse. Overall, serial samples and donor samples de-convoluted origins of relapse clone from all 20 patients. Among the 13 patients whose donor samples were sequenced, no mutation that was transferred from donor to recipient expanded at relapse. We then assessed whether the mutation status at pre- and post-HCT has any impact on OS and relapse after HCT. With a follow-up duration of 6.9 years, patients with VAF ≥20% at day 21 in any gene showed worse OS (HR 2.9, p=0.006) as well as increased risk of relapse (HR 5.3, p=0.0003) (Fig C-D). Multivariate analyses verified that increased VAF (≥20%) at day 21 was considered an independent, prognostic risk factor for OS (HR 3.2, p=0.003). Non-relapse mortality did not show significant difference (p=0.3). Thirteen patients carried 20 mutations at day 21 (≥20%), 16 of which originated from the initial AML clone. Notably, 9 of these mutations (from 9 patients) were in DNMT3A, where one of them was transferred into the donor clone after HCT (donor VAF: 8.18% VAF at day 21: 3.42%, non-relapse case). Mutation status at any other time points in any gene, defined with a hard cut-off (VAF >2%), was not significantly associated with overall survival nor relapse incidence after HCT.

Summary/Conclusions: This study revealed origins of mutations detected at post-HCT, which revealed that 3.8% of mutations assessed after HCT, within 21 days, with low VAF (0.2%) can be used to predict relapse after HCT, illustrating the value of longitudinal NGS-based monitoring strategies for AML patients after allo-HCT.

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IBRUTINIB FOR CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER FAILURE OF FRONTLINE CORTIROCORTICOSTEROIDS: RESULTS OF A MULTICENTER OPEN-LABEL PHASE 2 STUDY
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Background: There are no approved therapies for chronic GVHD (cGVHD) after failure of steroids. Both B and T cells play a role in the pathophysiology of cGVHD. In preclinical models,ibrutinib (ibr) reduced the severity of cGVHD by inhibition of Bruton’s tyrosine kinase (BTK) and interleukin-2–inducible T-cell kinase (ITK).

Aims: This phase 2 study evaluated the efficacy and safety of ibr in patients (pts) with steroid-dependent/refractory cGVHD in need of additional therapy.

Methods: Eligible pts had ≤3 prior regimens for cGVHD and either >25% body surface area erythematous rash or a NIH mouth score >4. Informed consent was obtained from all pts. Pts were treated with ibr 420mg/d until cGVHD progression or unacceptable toxicity. The primary end point was cGVHD response based on 2005 NIH consensus response criteria. Secondary end points included rate of sustained response, change in Lee cGVHD symptom score from baseline after ≥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 safety profile showed no major ibr-related malignancies and those seen in cGVHD pts on concomitant steroids. Responses in this pretreated, high-risk population support study of ibr for frontline treatment of cGVHD.

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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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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/PB), cytogenetic group, patient and donor CMV serology status

Results: We identified 2654 pts (haplo=185; MSD=2469) for int-AML (HAPLO=122; MSD=1888) or high-risk AML (HAPLO=63; MSD=581). Median follow up was 17 months (range 0-85 months) after HCT. Among all HAPLO recipients, 74% received PTCY and 26% ATG. Conditioning regimen was myeloablative in 50% vs 52% (p=0.52) of HAPLO and MSD pts, respectively. 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 aGVHD and cGVHD was 29% vs 20% (p<0.03) and 30% vs 36% (p<0.02) in HAPLO and MSD pts, respectively. At 2 years, NRM and RI were 26% vs 10% (p<0.01) and 17% vs 20% (p<0.52) in HAPLO and MSD pts, respectively. 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/PB), cytogenetic group, patient and donor CMV serology status

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the standard of care for patients (pts) with intermediate (int-) and high-risk AML. In pts lacking matched sibling (MSD), 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 MSD

Methods: Included were adults with AML in first CR undergoing transplantation from HAPLO vs MSD 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 MSD 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/PB), cytogenetic group, patient and donor CMV serology status
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 48% vs 40% (p=0.17). In multivariate analysis, risk of grade IV aGVHD (HR: 2.20; 95% CI: 1.20-3.74; p<0.01) was increased after Haplo as compared to MSD and no difference was observed in LFS, OS and GRFS, respectively. Conditioning regimen was associated with lower NRM and higher GRFS, while younger age and donor CMV status was associated with lower RI, higher LFS and OS. Results were confirmed in the analysis combining with the the propensity score technique as for RI, NRM, LFS and 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

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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 depletes 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 iCas9p9 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 iCas9p9 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. CD3+/CD19+ T-cells can be tracked by flow cytometry.

Aims: This study was performed to evaluate both safety and efficacy of BPX-501 T cell infusion post αβ T-cell depleted haplo HSCT in pediatric patients with high risk ALL and AML in CR1 and CR2.

Methods: A prospective Phase II 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.

Summary/Conclusions: Engraftment was brisk and T cell recovery normalized by 6 months. Overall incidence of severe aGVHD was low and the safety switch was successfully activated with rimiducid infusion. Cumulative incidence of NRM compares favorably to historic controls at the lead center, where a value of of 2.4% for matched related donors (MR), 11.8% for matched unrelated donors (MUD) and 5% for αβ T cell depletion haplo HSCT (Haplo αβ) without BPX-501 infusion was recorded (Bertainia, 2015 ASH). The cumulative incidence of relapse was 12.0% for BPX-501, 32.3% for MR, 22.2% for MUDs and 21.9% Haplo-αβ. Disease-free survival in the BPX-501 treated patients was 84.2% compared to 65.4% for MR, 66.1% for MUDs and 73.1% for Haplo-αβ. However, length of follow-up on the control cohorts differed from that of BPX-501 treated patients. These data suggest that BPX-501 T cells modified with the iCas9p9 safety switch, infused after selective αβ T-cell depletes, are safe and result in a rapid immune reconstitution and a potentially stronger GvL effect in children with high-risk leukemia who lack a matched donor.

Figure 1.

Result: 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 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 transplant 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.

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 sequenced for mutations in 54 genes. We used a Cox multivariate model and result in a rapid immune reconstitution and a potentially stronger GvL effect.
Bone marrow failure and PNH

S496
HEREDITARY HEMATOLOGIC MALIGNANCIES: GENETIC COUNSELING IMPLEMENTATION IN A LARGE LEUKEMIA CENTER
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Background: Hematologic malignancies have rarely been targets for genetic evaluation, even in familial cases. Over the past decade, more than 12 genes have been identified to cause inherited predispositions to hematologic malignancies. Genetic counseling, testing, and surveillance protocols for these families are not well-established. Additionally, many families with high incidence of blood cancers do not have described syndromes suggesting additional genes remain to be identified.

Aims: To identify individuals with inherited susceptibilities to hematologic malignancies, the Hereditary Hematologic Malignancy Clinic (HHMC) was established in April 2014 at The University of Texas M. D. Anderson Cancer Center. The clinic serves a large group of patients with hematologic malignancies suspected to have inherited predisposition syndromes.

Methods: Individuals were referred to the HHMC for several indications: (1) bone marrow failure/aplastic anemia/hypocellular MDS, (2) personal history of hematologic malignancy with <1 first-degree relative or ≥2 second-degree relatives with hematologic malignancy, (3) personal history of multiple primary cancers, (4) germline evaluation of presumed somatic mutations identified on next-generation leukemia prognostication panels, (5) management and/or surveillance of a previously-identified genetic syndrome, or (6) solid tumor hereditary syndromes with active hematologic malignancy. Over the past 3 years, 152 probands were evaluated (n=152). Skin biopsies were performed to obtain germline DNA, and next-generation sequencing approaches on both a clinical and research basis were utilized.

Results: Clinical genetic testing was performed in 97/152 individuals (64%). Research testing was performed in 46/152 (30%), particularly in patients negative for known susceptibility genes or without features suggestive of a clinical syndrome. Nine (6%) individuals did not undergo genetic testing. Clinical testing identified 23/97 (24%) individuals with a germline susceptibility to hematologic malignancy. Seven probands (7%) were identified to have RUNX1 mutations associated with familial platelet disorder with myeloid malignancy (FPD-AML). Six (6%) were identified to have the telomere disorder dyskeratosis congenita; only one of them met clinical diagnostic criteria with the “classic triad” of symptoms. Three (3%) patients were identified to have Li-Fraumeni syndrome due to constitutional TP53 mutations. Two adults (2%) were diagnosed with Diamond-Blackfan anemia, both of these individuals developed adult-onset myelodysplastic syndrome after a long latency period and prior spontaneous remission of their childhood anemia. Two young adults (2%) with Fanconi anemia were diagnosed, and one patient each with DDXX47 mutation and CBL (Noo-nan-like syndrome with JMML) were identified. Counseling, testing, and surveillance of identified mutation carriers in many affected families is ongoing.

Summary/Conclusions: Individuals with hereditary susceptibilities to hematologic malignancies are not as rare as previously thought. Clinical evaluation of these patients through genetic counseling and testing is high yield for identified at-risk families. Research-based sequencing for novel mutations is indicated and ongoing.

S497
SECONDARY LEUKEMIAS IN GENETIC SUBTYPES OF CONGENITAL NEUTROPENIA (ELANE, HAX1, WASP, G6PC3, ETC.): A LONG-TERM 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 have been followed by causal and/or molecular analysis for respective genetic subtypes: ELANE, HAX1, G6PT, G6PC3, WAS, SBDS, TAZ1 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 TAZ1 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 secondary AML. 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.

Summary/Conclusions: The incidence of secondary AML reflects the genetic heterogeneity of CN.

Table 1.

<table>
<thead>
<tr>
<th>Gene Mutation</th>
<th>Patients</th>
<th>MDS/Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CN</td>
<td>445</td>
<td>49 (11.0)</td>
</tr>
<tr>
<td>ELANE</td>
<td>118</td>
<td>17 (14.5)</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>SLCN4A4</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>35</td>
<td>16 (11.8)</td>
</tr>
<tr>
<td>unclassified</td>
<td>91</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Total CYN</td>
<td>91</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>ELANE CyN</td>
<td>43</td>
<td>1 (2.3)</td>
</tr>
</tbody>
</table>

*Gene mutations without leukemia: (G6PC3 n=9, TAZ1 n=5, p14 n=4, digenic mutations n=4, COH1 n=4, CXCR4 n=3, germline extracellular CSF3R n=2, C16orf57 n=2, Pearson syndrome n=2, LVST 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.

S498
EFFECT OF ECULIZUMAB 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 (HDA).

Methods: Patients enrolled in the Registry as of December 5, 2016, were stratified by HDA and ecu treatment status into 4 groups: HDA/ecu-treated; HDA/never ecu-treated; no-HDA/ecu-treated; no-HDA/never ecu-treated. HDA is defined as lactate dehydrogenase (LDH) ratio ≥1.5x upper limit of normal within 6 months of baseline and history of any of the following: fatigue, hemoglobinuria, abdominal pain, 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 last follow-up. Outcomes include changes from baseline to last follow-up in LDH ratio, GPI-deficient granulocytes, red blood cell transfusions received, MAVE, and Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue score in patients with at least 6 months of follow-up.
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=111; 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 euc-treated patients for both the HDA and no-HDA groups (see Table). Results for changes from baseline to last follow-up in outcomes of interest are summarized in the Table. Data show that patients in the euc-treated cohort had high burden of disease at baseline. Specifically, in the HDA population, a higher proportion of ecu-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 MAVE at baseline, treatment with ecu was associated with meaningful improvements in mean (standard deviation [SD]) reduction from baseline in LDH ratio (-5.0 [3.7] vs -0.4 [2.3]) and proportion of red blood cell transfusion-free patients (37.6% vs 15.8%). The FACIT-Fatigue data, while limited, showed the HDA/euc-treated group experienced a greater mean (SD) score improvement than the HDA/never 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 CELLS USING CRISPR-CAS9

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Background: Thrombopoietin (Tpo) and its receptor, Mpl, are the principal regulators of early/late thrombopoiesis and hematopoietic stem cells maintenance. Mutations in MPL can drastically impair its function and be a contributing factor in multiple hematologic malignancies, including congenital amegakaryocytic thrombocytopenia (CMT). CMT is a rare inherited syndrome characterized by thrombocytopenia at birth, progressing to bone marrow failure and pancytopenia. The functional impact of CMT mutations on Mpl is yet to be determined. Here we report unique familial cases of CAMT presenting with a previous G117T (K39N or Baltimore) mutation.

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 erythocyte 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 PNH erythrocytes (Type III) in a dose-dependent manner. More broadly, this series of molecules has been profiled by in vitro and in vivo ADME disposition studies and exhibits oral bioavailability (%F~30-50) in pre-clinical species.

Summary/Conclusions: The results presented here highlight, for the first time, the development of an orally potent small molecule inhibitor of C5. The demonstration of an orally available complement C5 inhibitor has the potential to provide a new therapeutic modality to treat both rare and common conditions where terminal complement cascade inhibition is desired.
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 following by thalidomide maintenance (MPT-T) versus melphalan, prednisolone and lenalidomide followed by lenalidomide maintenance (MPR-R) (Zweegman S et al. Blood 2016;127(9):1109-1116). As not only efficacy but also potential toxicity affecting quality of life (QoL) guides the choice of treatment, health-related (HR) QoL is important. Aims: To evaluate the HRQoL results of the HOVON87/NMSG18 study. Methods: Two validated HRQoL instruments (EORTC QLQ-C30 and MY20) were assessed at baseline, after 3 and 9 induction cycles (3ID and 9ID) and after 6 and 12 months of maintenance therapy (6MT and 12MT). The subscales global QoL, physical functioning, pain, fatigue, constipation, diarrhea, nausea/vomiting, insomnia, disease symptoms, side effects of treatment and neuropathy were described in previous literature and were used to determine clinically relevant difference in HRQoL score at each time point between thalidomide and lenalidomide. To determine clinically relevant difference in HRQoL score at each time point, the minimal important difference (MID) was used. In MPT-T MID was reached for the following subscales: global QoL decreased after 9ID and 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 more patients (MID >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 OBINUTUZUMAB-BASED AND RITUXIMAB-BASED THERAPY IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED INDOLENT NON-HODGKIN LYMPHOMA


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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.

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 (NCT01332068) is an open-label, randomized Phase III study of obinutzumab (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.

**Summary/Conclusions:** Of 1202 FL pts randomized (median age, 59 yrs; 53.2% female; median observation time, 34.5 mo [range 0–54.5], 586/901 [82.5%; G-chemo] and 550/601 [91.5%; R-chemo] completed all FACT-Lym scales at baseline. Baseline demographic and disease characteristics were balanced between arms. At baseline, mean HRQoL scores were similar in the two treatment arms, with all pts having some impairment of physical function, functional wellbeing, emotional and social function. Over the course of treatment, mean HRQoL was similar in the two treatment arms. From end of induction onwards, pts in both arms experienced clinically meaningful improvements from baseline in LYMS scores (Figure), and the summary scales that included this subscale (TOI, Lym-Tot). On each summary scale, ~50% of patients in each arm reported clinically meaningful improvements from baseline in HRQoL scales 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 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 clinically meaningful improvements from baseline in LYMS Total. On each summary scale, ~50% of patients in each arm reported clinically meaningful improvements from baseline in LYMS scores (Figure), and the summary scales that included this subscale (TOI, Lym-Tot). On each summary scale, ~50% of patients in each arm reported clinically meaningful improvements from baseline 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 physical and functional wellbeing, and disease- and 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.

**Figure 1.**

**Methods:** Enrolled pts were aged ≥18 years with documented, previously untreated FL (grades 1-3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG performance status 0-2, and requiring treatment according to GELF criteria. Pts were randomized 1:1 to R 375mg/m² on day (D) 1 of each cycle (C) or G 1000mg on D1, 8, and 15 of C1 and D1 of C2-8, for 6 or 8 cycles depending on chemo (CHOP, 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 wellbeing, 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 clinically meaningful improvements from baseline in LYMS Total. On each summary scale, ~50% of patients in each arm reported clinically meaningful improvements from baseline 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 physical and functional wellbeing, and disease- and 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.
THE SIMM STUDY: SURVEY OF INTEGRATIVE MEDICINE IN MYELOPROLIFERATIVE NEOPLASMS

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Aims: To investigate integrative therapy utilization association with 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.

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 with 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), strength training (p=0.001), breathing exercises (p=0.001), and support groups (p=0.001). Depression (PHQ-9 total >3 category) was lower in aerobic activity group (p=0.001), yoga (p=0.001), strength training (p=0.001), and meditation (p=0.2). Fatigue was lower in aerobic activity (p<0.001), massage (p=0.04), strength training (p=0.001), breathing exercises (p<0.001), and support groups (p=0.001).

Results: Patients: A total of 1087 patient surveys were consented. Of these, 888 had 10 or more responses. There were 338 essential thrombocytosis (ET), 188 myelofibrosis (MF), 315 polycythemia vera (PV), and 17 other. In MF: MPN-SAF 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), strength training (p=0.001), breathing exercises (p=0.001), and support groups (p=0.001). Depression (PHQ-9 total >3 category) was lower in aerobic activity group (p=0.001), yoga (p=0.001), strength training (p=0.001), and meditation (p=0.2). Fatigue was lower in aerobic activity (p<0.001), massage (p=0.04), strength training (p=0.001), breathing exercises (p<0.001), and support groups (p=0.001). In subgroup analysis, ET and PV patients had lower symptom burden (MPN-SAF TSS with aerobic activity (p<0.001), massage (p=0.01, 0.02), and strength training (p=0.03, 0.02). Support groups were found to be associated with lower symptoms in ET patients (p=0.03). In MF, breathing exercises (p<0.001) and support groups (p=0.03) were associated with lower symptom burden. See Table #1.

Table 1.

<table>
<thead>
<tr>
<th>MPN-SAF TSS</th>
<th>PHQ-9</th>
<th>BFI Usual</th>
<th>Overall N=488</th>
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<tr>
<td>ET 152</td>
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<tr>
<td>PV 315</td>
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<td>MF 121</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.
**POSTER SESSIONS II**

**Acute lymphoblastic leukemia - Biology 2**

**P506**

**T CELL EXHAUSTION CHARACTERIZED BY COMPROMISED MH CLASS I AND II RESTRICTED CYTOTOXIC ACTIVITY ASSOCIATES WITH ACUTE BLYMPHOCYTIC LEUKEMIA RELAPSE AFTER ALLOGrafted HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background:** B cell acute lymphoblastic leukemia (B-ALL) relapse contributes to the predominant mortality after allogeneic hematopoietic stem cell transplantation (allo-H SCT). However, the mechanism of B-ALL relapse after allo-H SCT remains unknown. Eradication of leukemia in allo-H SCT 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-H SCT, whether T cell exhaustion is involved in B-ALL relapse after allo-H SCT remains unknown.

**Aims:** To evaluate whether T cell exhaustion is involved in B-ALL relapse after allo-H SCT, and to investigate the correlation of inhibitory ligands on leukemic cells, leukemic load and T cell exhaustion, as well as the impact of treatment outcome on T cell exhaustion.

**Methods:** Our study enrolled 18 B-ALL patients who underwent first hematologic relapse after allo-H SCT and 18 matched B-ALL patients in remission (without minimal residual disease MRD) and 14 healthy donors from April 2016 to November 2016 at the Peking University People’s Hospital. In the hematologic transplant protocol and post-transplant time were matched in relapsed and non-relapsed patients. Post-transplant time were matched as follows: ±14 days within 12 months ±1 months from 12 to 18 months, ±3 months from 18 to 36 months, ±12 months over 3 years. Extra-medullary relapse were excluded in our study. All patients had achieved full donor chimerism before relapse or bone marrow collection. Peripheral blood (PB) were collected at the same day of bone marrow collection in relapsed patients. For patients who received induction therapy, we prospectively collected BM at least once after therapy. Sample collection was performed after patients was informed consent and approval by the institutional Human Ethics Review Committee of Peking University People’s Hospital in accordance with the Declaration of Helsinki. phenotypic and functional studies of T cells in those patients were performed using multi-color flow cytometry.

**Results:** In the current study, we observed that increased co-expression of PD-1 and Tim-3 was observed in both CD4+ and CD8+ T cells in relapse settings. Moreover, both CD4+ and CD8+ T cells exhibited compromised proliferative capacity, cytokine production and cytotoxic potentials such as degranulation and granzyme B production (preferentially on CD4+ T cells) in relapsed patients. In addition, T cells in the tumor live are more easily exhausted than those in peripheral blood. Reversal of T cell exhaustion was associated with effective anti-leukemic response in relapsed patients who underwent re-induction therapy.

**Summary/Conclusions:** In conclusion, our study suggested that T cells experienced expression of a comprehensive, functional impairment in B-ALL relapse settings after allo-H SCT and reversal of T cell exhaustion was associated with effective anti-leukemic responses. These results also provide a foundation for the development of novel effective leukemia therapeutics, such as anti-PD-1 or PD-L1 therapy, by targeting T cell exhaustion.

**P507**

**RUXOLITINIB/NILOTINIB CO-TREATMENT BETTER INHIBITS LEUKEMIA-PROPAGATING CELLS IN PHILADELPHIA CHROMOSOME-POSITIVE ALL**

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**Background:** Relapse remains the major cause of treatment failure in patients with childhood acute lymphoblastic leukemia (Ph-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 immune-compromised mice. Using an anti-CD122-conditioned NOD/SCID humanized xenograft model, we investigated whether selective BCR-ABL/JAK2 dual inhibition therapy could more effectively eliminate LPCs in vitro and in humanized Ph+ALL mice.

**Methods:** Using RNA-seq and qRT-PCR, we found that JAK2 was more highly expressed in the sorted LPCs than in the cells of other phenotypes in patients with de novo Ph+ALL in vitro study, cotreatment with nilotinib and ruxolitinib induced significantly higher levels of apoptosis in LPCs. In humanized Ph+ALL mice model, treatment with the nilotinib and ruxolitinib combination, compared with either ruxolitinib or TKIs alone, led to the most significant reduction in human Ph+ALL engraftment in the recipients. Further evidence that the most effective anti-LPCs effect occurred with the combination treatment was derived by the engagement analysis of BCR-ABL expressing cells using a rQ-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 decrease in expression of phospho-CrKL, JAK2 and STAT5 activities at the molecular level.

**Summary/Conclusions:** JAK2 was more highly expressed in the sorted LPCs than in other cell phenotypes in patients with de novo Ph+ALL. Furthermore, selective BCR-ABL/JAK2 dual inhibition with nilotinib/ruxolitinib more effectively eliminated LPCs than either ruxolitinib or TKIs alone. This 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.
related with ABT-199 sensitivity (k = 0.71, p = 0.00), highlighting the importance of functional assessment of the direct target molecule and other resistance mediating molecules. In line, MCL-1 knockout in two ABT-199-resistant cell lines led to sensitization towards ABT-199, however, resulted in different effects of sensitization, emphasizing that ABT-199 resistance is determined by the interplay of several apoptosis regulators. Therefore, we characterized the functional dependence of pdx leukemias on anti-apoptotic BCL-2 family members. Mitochondrial dependence on BCL-2 (mitochondrial priming by the BAD-peptide measuring BCL-2, BCL-XL and BCL-W, and subtracting the response to the HRK-peptide measuring BCL-XL) was found to be tightly correlated with ABT-199 sensitivity. In contrast, ABT-199-resistant samples were characterized by low BCL-2-dependence and addiction to other BCL-2 family members, including BCL-XL or MCL-1. Finally, we evaluated prediction of in vivo ABT-199 sensitivity in a pre-clinical ALL pdx mouse model by functional BH3 profiling. Strikingly, high mitochondrial BCL-2-dependency was clearly associated with prolonged leukemia-free survival upon ABT-199-therapy (two pdxs, log rank p = 0.0035 and <0.0001), in contrast to another leukemia with low BCL-2-dependence and in vivo ABT-199 resistance (log rank p = 0.144).

Summary/Conclusions: SCP-ALL displays heterogeneous ABT-199 sensitivities characterized by the level of the target molecule but also other interacting regulators. Functionally, mitochondrial BCL-2-dependency assessed by the BH3 profiling assay is clearly associated with ABT-199 sensitivity. Importantly, in vivo anti-leukemia activity of ABT-199 therapy in individual pdx leukemias is predicted by mitochondrial BCL-2-dependence, emphasizing the utility of identification of patients and guidance of future clinical application by functional assessment of apoptosis signaling.

**P510**

**A BILINEAL ACUTE LYMPHOBLASTIC LEUKEMIA ORIGINATING AT A COMMON LYMPHOID PROGENITOR**

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Background: Genetic mutations are crucial events during leukemogenesis and provide specific markers for backtracking the cellular origin of acute leukemias up to immature uni- or multi-potent progenitor cells in the hierarchy of the hematopoietic system.

Aims: To characterize the clonal architecture and cell of origin in a case of B-cell ALL and B-ALL.

Methods: Bone marrow cells obtained at diagnosis were used for all studies. Immunophenotyping was done by flow cytometry. T- and B-cell leukemic purification was performed by immunomagnetics methods and DNA extracted afterward. TCR-gamma gene rearrangement was studied in T- and B-leukemic cells independently by PCR spectratyping. Somatic mutations in purified T- and B-leukemic cells were identified by deep-sequencing using a panel of 160 genes frequently mutated in cancer (Human comprehensive cancer panel, Qiagen). Mutations were validated by Sanger sequencing. Myeloid and erythroid clonogenic progenitors were isolated from methylcellulose cultures, DNA extracted, and assessed for the presence of the H3F3A p.K28N mutation by Sanger sequencing.

Results: The patient was a 10 years old boy. At diagnosis, the bone marrow was infiltrated by 60% leukemic cells, with 2 immunophenotypically different populations: a common B-ALL (54%) and a pro-T-ALL (6%). The patient showed a mediastinal mass in the chest X-ray that was confirmed by computed tomography. The TCR-gamma rearrangement was detected in purified (>95% pure) T-ALL and B-ALL cells, suggesting a common origin for both leukemic subpopulations. The B-ALL cells presented a c.35G>A p.G12D mutation in the KRAS gene, absent in the T-ALL. The T-ALL cells presented a c.35G>A (p.G12D) mutation in the NRAS gene, absent in the B-ALL. A c.1126_1127insTAGA (p.P376Lfs*10) mutation in the WT1 gene was also detected only in the T-ALL. A c.84G>T (p.K28N) mutation in the H3F3A gene was detected in both the B-ALL and T-ALL subpopulations, confirming the involvement of a Common Lymphoid Progenitor in the process of leukemogenesis. The presence of the H3F3A p.K28N mutation in the myeloid compartment would point to a multipotent myeloid-lymphoid rather than a lymphoid-restricted progenitor as the cell origin of the leukemia. Therefore, we cultured 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-GM) were present 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 (CSRP2) TRANSCRIPT LEVELS CORRELATE 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. Lymphoblastic leukemia remains a difficult disease to treat, and few biomarkers in B-ALL especially in those with normal cytogenetics and studying their clinical significance and biological function will be helpful for risk-stratification, treatment decision and targeted therapy. CSRP2 (cysteine and glycine rich protein 2) maps to chromosome subband 12q21.1, which is frequently abnormal in diverse cancers. Increased CSRP2 transcript levels were associated with de-differentiation in hematopoietic cell carcinoma and CSRP2 was predicted to be a new invadopodia actin bundling factor that critically promotes breast cancer cell invasion and metastasis. However, the clinical significance and biological function of CSRP2 in B-cell ALL remains unknown.
Aims: To identify novel biomarkers in B-cell ALL based on bioinformatics analyses; to examine the expression and clinical significance of CSRP2 in adults with B-ALL; to explore effects of CSRP2 on biological function of B-cell ALL.

Methods: We did bio-informatics analyses to identify mRNA transcripts aberrantly-expressed in B-cell ALL. RT-qPCR (real-time quantitative polymerase chain reaction) was used to examine CSRP2 transcript levels in bone marrow samples from 236 adults with B-cell ALL compared with samples from normal. A prognostic value was assessed in 168 subjects. CSRP2-knockdown and CSRP2-over-expression cell models were constructed to study the biological function of CSRP2 in B-cell ALL.

Results: We selected 9 candidate genes for validation 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 with 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 multivariable 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: 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.

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THEРАPEUTIC TARGETING OF PRE-Б-CELL RECEPTOR SIGNALLING IN CHILDHOOD ACUTE LYMPHOBlastic LEUKEMIA

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Background: Acute lymphoblastic leukaemia (ALL) is the most common malignancy in children and adolescents and relapsed ALL remains one of the leading causes of cancer-related deaths in children. Components of the precursor-B cell receptor (Pre-BCR) signalling pathway are hijacked in ALL cells and this dependence may be therapeutically targeted. A number of tyrosine kinase inhibitors (TKIs) targeting effectors of this signalling pathway are showing great promise in the clinic and warrant preclinical evaluation in paediatric ALL. They include Dasatinib (BCR-ABL/SRC inhibitor), Fostamatinib R406 (SYK inhibitor), Ibrutinib (BTK inhibitor) and CAL-101 (PI3K-δ inhibitor).

Aims: To preclinically evaluate these candidate TKIs, as novel, targeted drugs for children who 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 (μH, VpreB and A5) and functionality using a Calcium flux assay were detected by Flow cytometry. Intracellular phospho-flow cytometry was used to detect constitutive phosphorylation and activation in response to anti-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 the expression of CSRP2, Lzdn, GZM and IL15, GR expression and phosphorylation were detected by western blotting.

Results: ALL cell lines were modestly sensitive to Dasatinib (mean G50 53.3 μM, range 2.45-12.5 μM) and R406 (mean G50 4.32 μM, range 2.88-5.83 μM). However, cells were resistant to Ibrutinib (mean G50 15.9 μM, range 11.47-18.3 μM) and CAL-101 (mean G50 52.08 μM, range 25 μM-77.83 μM). Cell cycle arrest and significant apoptosis was seen with R406 and Ibrutinib treatment, while Dasatinib and CAL-101 were cytostatic, causing G1 arrest with no substantial cell death. Pharmacodynamic assays confirmed inhibition of the relevant drug targets, PDX cells showed greater sensitivity than the cell lines to Dasatinib (4 out of 16 patient samples <0.5μM), R406 (7 out of 16 patient samples <0.5μM), Ibrutinib (3 out of 15 patient samples <8.5μM) and CAL-101 (3 out of 15 patient samples <2μM). Pre-BCR positive ALL cell lines and PDX cells were sensitive to R406 and Dasatinib, with a Ph+ PDX confirming the sensitivity to the latter. Combining TKIs with the glucocorticoid (GC), Dexamethasone showed 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.

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BMP-4 LEVELS IN CHILDHOOD B-ALL OF LOW-/INTERMEDIATE-RISK GROUPS IDENTIFY CHILDREN WITH POOR OUTCOME

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Background: Leukemic relapses among children with acute lymphoblastic leukaemia (ALL) from low/intermediate-risk groups is a challenge for the cure of this disease. New biomarkers are needed for identifying children at high risk of relapses. Bone Morphogenetic Proteins (BMPs) are multifunctional secreted growth factors that belong to the TGF-β superfamily and are well-known for their indispensable roles in vertebrate development. In the cellular context, BMPs regulate fundamental processes such as cell proliferation, differentiation, migration and survival. In last years, important new information has been generated on the contribution of BMP family members, such as BMP4, in cancer biology.

Aims: Here we have evaluated the relevance of BMP4 signaling in ALL.

Methods: The expression levels of BMP-4 related genes (bmp-4, and bmp-receptors, signaling mediators, inhibitors and targets) in ALL blasts obtained at the time of diagnosis (n=56), and the BMP-4 levels in central system fluid samples (CSF), were quantified by RT-qPCR or ELISA. The engrafting potential of primary ALL cells, exhibiting high or low BMP4 levels, were assessed in xenotransplantation experiments using unirradiated NSG mice.

Results: BMP4 was expressed at significantly higher levels in ALL blasts of children who later relapsed (17.78 versus 26.68, arbitrary units, AU, p<0.05). Relapses among children with high BMP-4 expression occurred significantly later than those with low BMP-4 expression (845 days versus 282 days, p<0.05). The difference in the cumulative incidence of relapses (CIR) was quasi-significant between both groups (p=0.031). The ratio Smad7:Smad1, suggesting inhibition of the Smad-dependent signaling pathway, was significantly higher in ALL blasts of children who later relapsed (14.33 versus 5.13, AU, p<0.05). CIR was significantly higher (p<0.05) in the group of children with the Smad-dependent pathway inhibited. All these differences were detected considering the whole population, as well as only the low/intermediate-risk groups. BMP4 levels were significantly higher in CSF samples of children with leukemic infiltration of the central nervous system (16pg/ml versus 3.4pg/ml, p<0.001), as well as in the group of children who relapsed (10.6 pg/ml versus 1.8 pg/ml, p<0.001). Hematopoietic engraftment (marrow, spleen and peripheral blood) and CNS leukaemia occurred only in ALL samples with high BMP4 levels. Even more, no signs of disease were detected in mice transplanted with primary ALL cells that expressed low levels of BMP4. In independent experiments, pharmacological blockade of the canonical BMP signaling pathway significantly decreased infiltration of CNS and consistently resulted in amelioration of clinical parameters including neurologic score.

The results indicate that high BMP4 levels are required for both bone marrow engraftment and CNS infiltration by B-ALL cells. BMP4 levels in leukemia cells could be a useful biomarker to identify children with poor outcome in the childhood B-ALL of low-/intermediate-risk groups. Furthermore, BMP4 could be a new therapeutic target to blockade leukemic CNS disease.

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TARGETING LOCALIZATION OF THE IL-7 RECEPTOR WITHIN LIPID RAFTS AS A THERAPEUTIC STRATEGY FOR T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: T-cell acute lymphoblastic leukaemia (T-ALL) is a hematological malignancy characterized by immature T-cell excessive proliferation. To achieve remission, patients typically undergo 2 years of chemotherapy, associated with acute and chronic side effects. To enable reduced chemotherapy intensity and
Acute lymphoblastic leukemia - Clinical 2

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SYSTEMATIC MRI SCREENING IDENTIFIES EXTENSIVE ASYMPTOMATIC OSTEONECROTIC LESIONS IN ADOLESCENTS WITH ALL - FIRST INTERIM FINDINGS OF THE OPAL TRIAL
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Background: Cure rates for acute lymphoblastic leukemia (ALL) have increased to ~90% in the last decades, but come at a high cost as a substantial proportion of these children sustain toxic side-effects. Osteonecrosis (ON) is one of the most common and debilitating side effects, which severely impacts quality of life.

Aims: To analyze whether systematic magnetic resonance imaging (MRI) screening of adolescents can identify those with asymptomatic ON stage I and II, who subsequently develop symptomatic ON.

Methods: Children diagnosed with ALL aged ≥10 years, who were enrolled in the Department of Pediatric Oncology at OPAL (osteonecrosis in pediatric patients with ALL or lymphoblastic lymphoma LBL) trial, were analyzed. Standardized MRI screening of the hips and knees was scheduled at diagnosis and 6, 9, 12, 15, 18 and 24 months into treatment. All patients were assessed according to a standardized case report form recording symptoms and activities of daily living and functional impairments of the hips and knees based on modified Harris Hip and Knee Society scores every 3 months from diagnosis to the end of antileukemic treatment.

Results: Between 03/2013-12/2016, 64 patients (pts) were enrolled, median age at ALL diagnosis was 15 years (range 10-17), median time under evaluation was 11 months (range 0-45). 31 (48.4%) pts were male, 33 (51.6%) female. 61 (95.3%) were diagnosed with ALL, 3 (4.7%) with LBL. 36 (56.2%) pts were treated according to the AIEOP-BFM 2009 trial, 25 (39.1%) pts to the CoALL-08-09 trial and 3 (4.7%) pts were enrolled in the NHL-BFM registry and treated accordingly. Until December 31st, 2016, 2 (3.1%) pts died treatment related, 4 (6.3%) underwent allogeneic stem cell transplantation, and 5 (7.8%) pts each relapsed while under treatment and dropped out for other reasons. Thus, so far, 166 MRIs comprising 644 joints could be evaluated. At initial diagnosis of the leukemia, MRI showed asymptomatic osteonecrotic lesions stage II or higher in 3 of 60 pts (5%), at 6 months in 7 of 34 pts (20.6%) osteonecrotic lesions, at 9 months in 14 of 23 pts (60.9%), at 12 months in 14 of 23 pts (60.9%), at 15 months in 3 of 11 pts (27.3%), at 18 months in 2 of 9 pts (22.2%), and at the end of treatment in 2 of 6 pts (33.3%). 11 (17.2%) pts developed symptomatic ON between 6 and 15 months from diagnosis (median 10 months). Of 23 pts, in whom screening MRI revealed ON stage II or higher, 11 pts (47.8%) subsequently developed symptomatic ON, whereas in all adolescents developing symptomatic ON MRI had previously shown signs of ON. Median volumes of epiphysseal necrosis in pts with ON stage II remaining asymptomatic were 0.6 ml (range 0.1-7.2) and in pts developing symptomatic ON 12.5 ml (range 12.0-13.9) in the hips and 2 ml (range 0.4-20.5) and 30.5 ml (range 18.3-57) in the knees respectively. Epiphysyeal involvement exceeded 30% in all symptomatic pts, but only in 2 pts remaining asymptomatic. With regard to the distribution pattern of ON, about twice as many knees as hips were affected by ON stage II or higher. MRI revealed ON stage III or higher in at least one joint in 12 pts (20%), predominantly in the knees. Radiological leukemic infiltration of bone detected by single screening MRI at diagnosis did not identify children at high risk of developing asymptomatic ON at six months into therapy or symptomatic ON anytime in the course of antileukemic treatment. These findings should be confirmed in larger patient numbers.

Summary/Conclusions: The first analysis of the OPAL trial shows that early MRI screening identifies extensive asymptomatic lesions in adolescents subsequently developing symptomatic ON.

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FINAL ANALYSIS OF A RANDOMIZED STUDY COMPARING PROPHYLACTIC AND MRD-TRIGGERED, PRE-EMPTIVE IMATINIB AFTER HSCT FOR PH+/BCR-ABL1 POSITIVE ALL: LONG-TERM PATIENT OUTCOME AND IMPLICATIONS OF MRD ANALYSIS
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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.

Summary/Conclusions: The study showed that PyQ delayed T-ALL progression in vivo 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 in vitro. Its delocalization leads to IL-7 signaling pathway inactivation, upregulation of BAD and BIM genes involved in apoptosis and T-ALL cells apoptosis. We furthermore assessed effect of PyQ on 10 samples of primary T-ALL blasts. All of them were sensitive to IL-7-dependent cell survival and revealed a marked response to PyQ treatment (Mean IC50=5.7 ng/mL). For this work, T-ALL cells were cocultured 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.
transplant or 2nd/3rd TKI remain to be determined. Validation in an independent dataset. Their applicability in the setting of RIC is IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/CR (69% at particular risk. Posttransplant 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, lack of prior treatment with IM or infections. Target dose of IM was 600mg/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), CR3 (n=1), unknown (n=1). Most pts. received a PBS, graft (n=71) 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 192d and 102d. Median follow-up of surviving pts. is 5.6 y (2.4-10.8) and 6.9 y (1.8-11). Relapse rate (14% vs. 18%), NRM (12% vs. 11%) and ongoing CR (69% vs. 71%) were not significantly different between arms. Probability of DFS and overall survival at 10 years was 64% vs 69% and 88% vs 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 3 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 (39% vs 24%, p=0.045) and inferior DFS (60% vs 71%, p=0.05) at 8 y. 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 10 y.

Summary/Conclusions: Post-HSCT intervention with prophylactic or pre-emptive IM is associated with a low relapse risk and excellent long-term survival and might be considered standard of care in Ph+ ALL pts. undergoing HSCT. BCR-ABL1 transcript levels prior to and early after SCT are predictive of outcome and identify a small subset of patients unlikely to benefit, emphasizing the need for rigorous MRD monitoring. The identified MRD thresholds should be validated in an independent dataset. Their applicability in the setting of RIC transplantation or 2nd/3rd TKI remain to be determined.

Background: Front-line imatinib (IM) plus chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) is standard therapy for patients (pts.) with Ph+ ALL. Relapse after HSCT remains a major cause of treatment failure, and pts. in whom BCR-ABL transcript levels prior to and early after SCT are detectable after HSCT are at particular risk. Posttransplant 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, lack of prior treatment with IM or infections. Target dose of IM was 600mg/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.

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Summary/Conclusions: Post-HSCT intervention with prophylactic or pre-emptive IM is associated with a low relapse risk and excellent long-term survival and might be considered standard of care in Ph+ ALL pts. undergoing HSCT. BCR-ABL1 transcript levels prior to and early after SCT are predictive of outcome and identify a small subset of patients unlikely to benefit, emphasizing the need for rigorous MRD monitoring. The identified MRD thresholds should be validated in an independent dataset. Their applicability in the setting of RIC transplantation or 2nd/3rd TKI remain to be determined.

Background: The CD19-targeted chimeric antigen receptor (CAR) T-cell therapy CTL019, an investigational therapy that uses reprogrammed cytotoxic T cells to recognize and eliminate target cells, has shown high response rates in clinical trials for pediatric/young adult R/R B-ALL. The safety profile in this population has been limited to a single-center trial.

Aims: To identify any new safety issues with CTL019 emerging from use in multi-center trials.

Methods: Pooled data from 2 single-arm, multicenter phase 2 trials of CTL019 therapy in pediatric/young adult patients (pts) with R/R B-ALL (NCT02435849 and NCT02228096) were used to further characterize the safety of CTL019.

Table 1.

Table 1. 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; 46%), hypotension (24%), decreased appetite (21%), increased AST (19%) and ALT (12%), hypoponinemia (16%), hypokalemia (13%), hypophosphatemia (11%), and pulmonary edema (10%). Rates of G3/4 AEs and SAEs decreased substantially >8 wk post infusion (10% and 3%, respectively) and <8 wk post infusion (10% and 3%, respectively). 21 pts died post infusion: 16 (76%) from B-cell acute lymphoblastic leukemia (B-ALL) 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...
twice as likely to develop G3/4 CRS than pts with <50% BM blasts (n=29) (53% vs 29%; P<0.001) (NCT01564784; Kantarjian NEJM 2016 [data cutoff date: Oct 2, 2014]).

Aims: To assess the impact of baseline karyotype on response and toxicities in R/R ALL patients receiving InO from the INO-VATE study.

Methods: Full study details have been previously published. At screening, karyotyping was performed locally; ≥20 metaphase count was recommended for cytogenetic analysis. Karyotypes were interpreted using the International System for Cytogenetic Nomenclature. CR/CRi and MRD negativity rates (defined as <0.01% bone marrow blasts as assessed by flow cytometry) were comparable using a cut-off of 0.1% for baseline karyotype and post-ASCT karyotype. Survival estimates were compared using a log-rank test. Data as of March 8, 2016, are presented. Informed consent was obtained from all patients. All analyses presented were not adjusted for multiple testing.

Results: Of 326 patients randomized, 284 had cytogenetic data at screening (87% of INO-treated patients). 21.3% had normal diploid karyotype (≥20 metaphases), 17.1% complex (≥20 metaphase abnormalities), 13.4% Philadelphia- chromosome positive (Ph+), 6.7% diploid (≥20 or unknown metaphases), 4.9% hyperdiploid (≥20 or unknown metaphases), 4.9% hypodiploid or near-triploid, 1.2% Del (9p), 16.5% other chromosomal abnormalities, and 12.2% missing. Of 164 INO-treated patients, CR/CRi rate was 73% (95% confidence interval [CI] 66–80; Table 1) and MRD negativity rate was 59% (95% CI, 51–67). With InO, CR/CRi and MRD negativity rates were similar between the various cytogenetic subgroups, except for signs of significantly higher rates of CR/CRi with InO compared to 22 metaphases), complex, other, and missing cytogenetic subgroups (P=0.015) and numerically higher in the other cytogenetic subgroups. With InO, more patients with diploid (≥20 metaphases) karyotype proceeded to stem cell transplant versus other cytogenetic subgroups. With InO, the duration of remission (DoR) was significantly different between cytogenetic subgroups (P<0.0001), with diploid (≥20 metaphases) and other subgroups having the longest median DoR numerically and MLL subgroup having the shortest median DoR numerically; no significant differences in DoR were seen between cytogenetic subgroups with SC (P=0.785). 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 the InO and SC arms, overall survival (OS) differences between cytogenetic subgroups were not significant (P=0.1629 and 0.3040, respectively); however, although not statistically significant based on 97.5% CI for hazard ratio (HR), OS was numerically longer (HR <1) with InO versus SC in diploid (≥20 metaphases), MLL, complex, other, and missing cytogenetic subgroups. Generally, adverse event profiles did not vary by cytogenetic subgroup.

Summary/Conclusions: In patients with diploid (≥20 metaphases), complex, other, and missing cytogenetic karyotypes, CR/CRi rates were significantly higher with InO versus SC (P=0.0063), with high-risk groups, other, and missing cytogenetic subgroups, OS favored InO versus SC, though not statistically significant. Safety profiles generally were similar to the overall study population.

Table 1.
A PHASE II STUDY WITH A SEQUENTIAL CLOFARBONE-CYCLOPHOSPHAMIDE COMBINATION SCHEDULE AS SALVAGE THERAPY FOR REFRACTORY AND RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA (R/R) IN ADULT PATIENTS

Background: Clonidine, a microparticle dispersions (CMD) formulation of clonidine, has shown promise as an adjuvant therapy for patients with relapsed/refractory lymphoma.

Methods: This was a multicenter, open-label, phase II study of clonidine-CMD in patients with relapsed/refractory lymphoma. Eligible patients included those who had undergone at least one line of prior therapy, with at least one prior line of chemotherapy. The primary endpoint was the overall response rate (ORR) at 24 weeks (both measurable and non-measurable disease were assessed). Secondary endpoints included progression-free survival (PFS), overall survival (OS), and safety.

Results: A total of 35 patients were enrolled in the study, with 30 patients achieving an ORR of 43% (95% CI: 27-60). The median PFS was 9 weeks (95% CI: 6-12) and the median OS was 37 weeks (95% CI: 27-51). The most common adverse events were nausea (71%), vomiting (63%), and dizziness (57%).

Summary/Conclusions: Clonidine-CMD showed promising activity as an adjuvant therapy for patients with relapsed/refractory lymphoma. Clinical benefit was observed across various subgroups, including those with high-risk disease. The safety profile was consistent with previous reports, with nausea, vomiting, and dizziness being the most common side effects. Further studies are needed to confirm these findings and explore the optimal use of this regimen.
affect adults aged >20 years (https://seer.cancer.gov). Adult patients (pts) with B cell 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 the 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, Eastern Cooperative Oncology Group status 0-1. Pts received 1 or 2 × 10^6 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 × 10^6 dose, and phase 1 was then expanded to 6 pts at the 2 × 10^6 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 × 10^6 CAR T cells/kg. Across all pts, the most common grade ≥3 adverse events were cytokopenia (80%), febrile neutropenia (50%), pyrexia (40%), and transaminisits (40%). Grade ≥3 CRS and neurologic events were reported in 40% and 10% 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). CAR T cells achieved peak expansion within two weeks of infusion. Of the 6 efficacy evaluable pts, 6 (75%) achieved remission (including CR and 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.

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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 adults aged >20 years (https://seer.cancer.gov). Adult patients (pts) with B cell 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 the 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, Eastern Cooperative Oncology Group status 0-1. Pts received 1 or 2 × 10^6 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 × 10^6 dose, and phase 1 was then expanded to 6 pts at the 2 × 10^6 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 × 10^6 CAR T cells/kg. Across all pts, the most common grade ≥3 adverse events were cytokopenia (80%), febrile neutropenia (50%), pyrexia (40%), and transaminisits (40%). Grade ≥3 CRS and neurologic events were reported in 40% and 10% 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). CAR T cells achieved peak expansion within two weeks of infusion. Of the 6 efficacy evaluable pts, 6 (75%) achieved remission (including CR and CRS after completion of the DLT cohort. Manufacturing was successful in all pts; most pts achieved a minimal residual disease-negative CR. These results demonstrate promising efficacy with a manageable safety profile. Based on these results, ZUMA-3 continues to enroll pts, adding measures to further enhance safety and with planned expansion to phase 2.

P524
EXPOSURE-ADJUSTED ADVERSE EVENT COMPARING BLINATU-MOMAB VS SOC 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-precursor 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-precur sor ALL (refractory to primary induction therapy or salvage therapy, first relapse <1 year, second or later relapse, or relapse after allogeneic hematopoietic stem cell transplantation) were randomized to receive either blinatumomab or SOC (1 of 4 predefined regimens). Blinatumomab was dosed by continuous intravenous infusion (4 weeks on/2 weeks off) for up to five induction cycles (9 patients) or until ≥1 of 7 cycle-specific exposure-adjusted adverse event cut-offs were reached (4 weeks on/8 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) and infection (858 vs 249) were also lower for blinatumomab than for SOC (p<0.0001). Exposure-adjusted event rates for neurologic events were 743 for SOC vs 472 for blinatumomab, with median time (range) to onset of 7 (1-43) days and 7 (1-190) days, respectively, and grade ≥3 cognitive dysfunction (SOC vs blinatumomab) were 0 vs 10 for blinatumomab. The most frequent AEs reported in patients treated with blinatumomab in both arms; CRS events in the blinatumomab arm decreased between cycle 1 and cycle 2 (14% vs 2%). The majority of fatal AEs were related to infection in both arms.
Table 1.

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Summary/Conclusions: In this study, blinatumomab showed an AE profile consistent with that previously reported for relapsed/refractory AML, including similar rates of manageable CRS and neurologic events. Exposure-adjusted event rates were generally higher in SOC vs blinatumomab, including for cytopenias and infections.

P525

FACTORS ASSOCIATED WITH STEM CELL TRANSPLANTATION OUTCOMES IN PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN VERSUS CONVENTIONAL CHEMOTHERAPY


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Background: Inotuzumab ozogamicin (InO) therapy in relapsed/refractory acute lymphoblastic leukemia (R/R ALL) resulted in superior complete remission (CR)/CR with incomplete hematologic recovery (CRi) rates compared to conventional chemotherapy (C) versions had comparable IC50s on human AML cell lines as free drugs (12–26 vs. 5–77 pM) and as CD33-targeting ADCs (0.7 µg/kg; TI of 950 vs. 190 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 linker was chosen as the lead compound, named IMGN779. In multiple species, IMGN779 had a consistent toxicity profile (mouse, rats and monkeys), producing reversible cytopenias with no or minor changes in transaminases and without histologic evidence of hepatotoxicity. Importantly, IMGN779 was highly active at a single dose 10 µg/kg (payload) in an MV4–11 (FLT3-ITD) disseminated AML xenograft model, producing a 90% increased life span, and was well-tolerated and highly active in repeat dosing regimens (10 or 30 µg/kg, qw x 3 and q3d x 3) in a HL60 AML xenograft model. Similarly, in a MV4–11 xenograft model, the continuous dosing (5 µg/kg, qw x 2 or q3d) 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.

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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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 toxicity profile across multiple species and in vivo activity disseminated AML models and in multi-dose regimens. IMGN779, the final ADC design, is comprised of an indolino-benzodiazepine mono-imine DNA-alkylating payload, DGN462, coupled by a cleavable N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (s-SPDB) linker to a CD33-targeting antibody.

Aims: Select the best ADC out of multiple preclinical anti-CD33 ADC candidates, and assess its activity in vitro and in vivo in AML models.

Methods: Unconjugated payloads were evaluated in vitro for cytotoxicity on human AML cell lines. Payloads were compared, as CD33-targeting conjugates, in vitro for cytotoxicity on human AML cell lines and in vivo for tolerability in mice and TI against 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 regimens in AML xenografts.

Results: First, we selected a high affinity antibody to CD33 with retained ADCC 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 IC50s on human AML cell lines as free drugs (12-26 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 5-fold higher MTD (maximum tolerated dose) in mice and 5-fold larger TI in AML xenograft models. 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 regimens in AML xenografts.

Summary/Conclusions: In this study, blinatumomab showed an AE profile consistent with that previously reported for relapsed/refractory AML, 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.

P527

THE MIXED LINEAGE LEUKEMIA FUSION PARTNER ENL RECRUTS 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 transformation. Interestingly, mutations in ENL have also been associated with a pediatric nephroblastoma. In its wild-type configuration ENL serves as scaffold factor in protein complexes that stimulate transcriptional elongation but, to clear Polycomb-induced transcriptional repression.
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 reader linked to a histone ubiquitination complex. The ability to bind PAF1 cooperatively with ENL recruitment permits ENL to regulate PAF1-mediated repression in an elongation reporter system and also during transformation of primary cells by MLL-ENL in vivo. Reactivation of polycomb by ENL was accompanied by ubiquitination of histone H2B, the hallmark activity of PAF1 allied enzymes. On a global scale, ENL recruiting PAF1/PAF1 motifs and associated mRNA-Seq data demonstrated that MLL-ENL targeted genes stood out with a supraphysiological accumulation of H2BUb accompanied by hyper-accelerated transcription rates. Interestingly, examination of Wilms tumor specific ENL mutants allowed to elucidate the underlying mechanism of the MLL-fusion induced ENL hyperactivity. Introduction of Wilms-specific ENL mini-mouse AML cell lines in vivo showed that MLL-ENL progression and H2BUb modification of Hoxa9 and Meis1, two sentinel loci for polycomb action. This was dependent on the conserved YEATS domain of ENL that operated as “switch” binding either histone H3 or PAF1 thus effectively regulating ENL function as anti-repressor or elongator factor, respectively. With this, we established a new role of ENL with PAF1 and thus perturbed proper silencing. This effect was intensified in an MLL-ENL fusion where MLL itself provided a constitutive tether to PAF1 effectively creating a “super-transcription factor” that constitutively combined anti-repression with elongation capabilities.

Summary/Conclusions: In summary, targeting histone ubiquitination may be an additional Achilles heel for mixed lineage leukemia that merits further investigation of therapeutic utility.

P528

PKC EPSILON SUPPORTS ACUTE MYELOID LEUKEMIA BY MAINTAINING MITOCHONDRIAL REDOX HOMEOSTASIS

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Background: Although numerous genetic mutations contribute to the etiology and pathophysiology of acute myeloid leukemia (AML), the molecular machinery that is not mutated but supports AML biology remains largely unknown. Several studies have shown that AML cells, irrespective of genetic sub-type, display an oxidized intracellular redox environment compared to their healthy counterparts. The redox environment of AML cells is largely due to the elevated 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 retrovirus expressing either PKCε or SOD2/Catalase were used for gain-of-function assays. Cytoplasmic and mitochondrial superoxides were measured using hydroxyl-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. In vitro and in vivo proliferation assays were performed through FACS-based purification of shRNA-expressing cells followed either by: 1) growth in cytokine-enriched media or 2) transplantaion into syngenic mice for survival analysis.

Results: We have discovered that inhibition of PKCε: 1) promoted the death of K562 AML cell lines in vitro 2) abrogated AML progression driven by MLL-AF9 in vivo (p=0.0014) and 3) obstructed the growth of 5 out of 7 PD-AML samples in vitro. At the molecular level, we observed that PKCε inhibition led to a significant and dose-dependent increase in mitochondrial-produced superoxides—a specific type of ROS. Moreover, we found that enforced expression of PKCε can protect AML cells from lethal effects of superoxide-inducing agents 2-thienyltrifluoroacetone and Antimycin A. To identify potential ROS-regulatory enzymes downstream of PKCε, we performed whole cell proteomics and found that the mitochondrial superoxide-neutralizing enzyme SOD2 is decreased in AML cells depleted of PKCε. Similar to PKCε inhibition, we also observed a growth inhibition of SOD2 reduced the expansion of AML cell lines and PD-AMLS in vitro as well as significantly extended the onset of MLL-AF9-driven AML in vivo (p=0.0042). Finally, we also found that enforced expression of SOD2 in tandem with another anti-oxidant enzyme Catalase, reverses the anti-leukemia effects of PKCε inhibition confirming that PKCε supports AML pathophysiology by maintaining mitochondrial redox homeostasis.

Summary/Conclusions: Our results indicate that PKCε and SOD2 regulate mitochondrial redox homeostasis to support AML cell survival and disease progression and thus may represent a foundation for designing and developing novel therapeutic strategies.

P529

Abstract withdrawn.

P530

ROLE OF SHP2 IN A MOUSE MODEL OF AML CARRYING FLT3-ITD ALONG WITH LOSS OF TET2

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Background: SHP2, a protein tyrosine phosphatase coded by Ptnp11, is an essential protein that integrates signals from several different tyrosine kinase receptors with the major intracellular signaling pathways such as ERK, PI3K and STAT pathways and regulates cell survival, proliferation and differentiation. One of the SHP2 dependent cytokine receptor kinase, FLT3 when mutated to be constitutively activated co-operates with other genetic lesions like loss of Tet2 and Dnmt3a leading to transformation of myeloproliferative neoplasm (MPN) to acute myeloid leukemia (AML) in mouse models. Tet2 and Dnmt3a are involved in regulation hematopoietic stem cell (HSC) self-renewal and differentiation programs through regulation of DNA methylation. One of their own each of them leads to MPN but when present in combination, leads to AML. These mouse models of AML have a more pronounced stem cell phenotype as compared to traditional chemically induced or FLT3 targeted kinase inhibitor. Aims: Inhibition of SHP2 catalytic activity by a small molecule allosteric inhibitor has been recently demonstrated to retard the growth of receptor tyrosine kinase driven malignancies. Therefore, we wanted to investigate the role of SHP2 in leukemogenesis driven by loss of an epigenetic regulator (Tet2) and aberrant cytokine receptor tyrosine kinase (FLT3-ITD) signaling.

Methods: Mice were intercrossed to generate Ptnp11F/FTet2-/-/Flt3ITD+/Mx1Cre+ or Ptnp11F/FTet2-/-/Flt3ITD+/Mx1Cre+ mice. Deletion of Ptnp11 was induced at 8-10 week of age by injecting poly IC and changes in the hematopoietic compartment were analyzed by flow cytometry. Cell autonomous and non-autonomous effects of Ptnp11 on leukemogenesis were also evaluated in transplantation models.

Results: After ploy IC induced deletion of Ptnp11 there was a significant difference in the median survival between leukemic mice with with deletion of Ptnp11 versus non-deleted (n=9). Though the Ptnp11 deleted leukemic mice showed almost complete loss of long term HSC with concomitant increase in short term proliferating HSC in the bone marrow, they were still able to home and engraft in lethally irradiated recipient mice. These results indicate that loss of Ptnp11 does not impair the engraftment of leukemic stem cells though in normal mice deletion of Ptnp11 impairs the ability to stem cells to home to bone marrow niche and engraft. Deletion of Ptnp11 in both primary mice and secondary recipients was also associated with deregulation of myeloid and lymphoid cell distribution both in the periphery and bone marrow. Mice with deletion of Ptnp11 in the context of Flt3ITD did not generate immature or mature B cells. The effects of Ptnp11 deletion were more severe in vivo as compared to mice that received Ptnp11 deleted cells or when Ptnp11 was deleted after transplantation suggesting a role for SHP2 function in the bone marrow microenvironment in this model of leukemogenesis.

Summary/Conclusions: SHP2 has been recognized as a proto-oncogene on the basis of its ability to induce hematological malignancies when it is constitutively active and loss of SHP2 catalytic activity is associated with inhibition of tyrosine kinase driven malignancies. Our results demonstrate that the role of SHP2 in AML is dependent upon the presence of other genetic mutations. SHP2 regulates AML with loss of Tet2 with concomitant expression of Flt3-ITD through influence on both leukemic cells and the bone marrow microenvironment.

P531

CLUSTER REGULATION OF RUNX FAMILY BY "GENE SWITCH" TRIGGERS A PROFOUND TUMOR REGRESSION OF DIVERSE ORIGINS

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Background: Although Run-related transcription factor 1 (RUNX1) has been generally considered to be a tumor suppressor, a growing body of evidence suggests its pro-oncogenic property in acute myeloid leukemia (AML).

Aims: Demonstrate the anti-tumor 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 regression in AML, 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 reversed 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 vivo data analysis and Chi-qaq 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.

Though 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 function. As expected, additional knockdown of RUNX2 and RUNX3 in RUNX1-depleted AML cells effectively suppressed their proliferations. Thus, the simultaneous targeting of RUNX members as a cluster provides more stringent control of leukemia cells. Finally, we examined the antitumor potency of CRoX-1-mediated cluster regulations of RUNX. CRoX-1 treatment was indeed highly effective against leukemia as well as dismal-prognostic solid tumors arising from diverse origins in vitro. Moreover, this reagent was exceptionally well-tolerated in mice and exhibited excellent efficacy against xenograft mice models of AML, acute lymphoblastic leukemia, lung and gastric cancers, extending their overall survival periods in vivo.

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 CD54+ and CD54- groups. Remarkably, the M4-like and CD54- 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 estimates that differentiated groups presented an enriched activity for PKA, MEK 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 importantly, 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 PKA, PKCD or MEK which make them more sensitive to the inhibitors PF03758309, trametinib or midostaurin than those in the non-differentiated sets. Our results, therefore, suggest that phosphoproteomics and mass cytometry signatures can help in the stratification of patients with AML, in order to define the clinical benefit of targeted therapies. These approaches can be applied to other tumors and other types of cancer.
Background: Based on the prognostic significance, as well as the association with certain biological and clinical features, acute myeloid leukemia (AML) with biallelic mutations in the 
CCAT/enhancer-binding protein-alpha (CEBPA)
 gene has been included as a distinct entity into the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. CEBPA
 mutations (19.5%) are among the most common mutations in de novo AML, with 60% of the patients (pts) carry biallelic mutations. Several studies showed that CEBPA
 occur almost mutually exclusive with regard to other AML associated gene mutations such as NPM1 or FLT3
 mutations. Recently, mutations in the tet oncogene family member 2 (TET2mut) were described as a frequent concurrent mutation of CEBPA
. Both genes are involved in the control of proliferation (CEBPA
) or differentiation (TET2mut) of myeloid progenitors. Preliminary data suggest that pts harboring the CEBPA
/TET2mut genotype have a significantly worse overall survival (OS).

Aims: To evaluate the frequency and the clinical impact of TET2mut within a large cohort of CEBPA
AML pts.

Methods: In total 200 AML pts (age 18 to 78 years) with CEBPA
(n=113) or CEBPA
single mutations (CEBPA
(n=87) were analysed for the presence of TET2mut. All pts were enrolled in one of 6 AMLSG treatment trials applying intensive therapy [AMLHD93 n=14; AMLHD98A (NCT00146120) n=53; AMLHD98B n=12]; AMLSG 07-04 (NCT00151242) n=74; AMLSG 06-04 (NCT00151255) n=25 and AMLSG 12-09 (NCT01180322) n=22]. TET2 mut screening was performed using a DNA-based PCR-assyay covering exons 3 to 9 followed by Sanger sequencing.

Results: In total we detected 52 TET2mut, 39 of the 200 pts (19.5%); In 16 pts TET2mut were limited within the cytogenetic intermediate-risk group (100%), and pts with TET2mut were significantly older than pts with TET2 wild-type (TET2wt, 58 vs 46, P<0.001). In addition, TET2mut were more frequent in secondary/therapy-related AML (P = 0.04), and there was a significant association with SRSF2 gene mutations (P = 0.01). With regard to outcome, pts with TET2mut had a significantly shorter event-free (EFS), relapse-free (RFS), and OS compared to TET2wt pts (P < 0.001, and P = 0.001, respectively). Event-free survival (EFS) decreased in a linear manner with increasing TET2mut within the subgroup of CEBPA
 (P = 0.003), indicating that a high TET2mut burden within the CEBPA
 gene might be associated with decreased survival. When we analyzed the impact of TET2mut within the subgroup of CEBPA
 pts (n=87), in this additional subgroup we found a significant association of TET2mut with older age (49 vs 46, P = 0.05), and an inferior EFS (P = 0.001), RFS (P = 0.003), and OS (P = 0.007). Finally, we analysed the impact of TET2mut within the subgroup of CEBPA
 (n=87). In this additional subgroup we found to be significantly associated with older age (65 vs 55, P = 0.001), and with SRSF2 mutations (P = 0.02). Clinically, pts with TET2mut had a shorter RFS (P = 0.02) and OS (P = 0.05), and trend a shorter EFS (P = 0.09).

Summary/Conclusions: In our study on a large cohort of CEBPA
AML pts we could confirm the high incidence of concomitant TET2mut (19.5%). Pts with concurrent TET2mut were significantly older and had an inferior outcome.

References:


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Background: Recent next-generation sequencing (NGS) studies have improved our understanding of the genomic landscape of CBF-AML (Faber 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, less is known about the impact of other known drivers, such as GFI1b, on the genomic architecture of related disease. Dasatinib targets the Src family tyrosine kinases with potential anti-leukemic activity, and is currently licensed for use in Philadelphia chromosome-positive chronic myeloid leukemia. However, the impact of Dasatinib on the GFI1b decoding of leukemic stem cells remains to be elucidated. As the impact of GFI1b remains elusive, AML with high-risk features such as co-mutation of NRAS and KIT remains challenging. In addition, the role of GFI1b in AML with different genetic backgrounds remains to be elucidated.

Aims: (i) to investigate the impact of GFI1b mutation status on the genomic landscape of AML patients; (ii) to determine whether the presence of GFI1b is associated with clinical outcome.

Methods: Whole-exome-sequencing (WES) was performed in paired diagnosis, remission and relapse samples of 38 patients with CBF-AML [inv(16), n=16; t(8;21), n=22] 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, NCT00580382; 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 SNOVs and sequencing artefacts. In sum, we identified 587 variants in 430 genes. At
diagnosis. 8.9 variants per patient were found as compared to 5.7 at relapse. 52% variants were present at diagnosis, 26% at relapse only, and 22% were present at both, diagnosis and relapse. With regard to the most commonly altered signaling genes KIT and NRAS we found the following pattern: The median VAF at diagnosis was 23% and 26% for KIT and NRAS, respectively. Of note, the initial KIT and NRAS clone was lost (VAF <5%) in 71% (exon 17, n = 9; exon 8, n = 2; exon 11, n = 1) and 100% of cases (exon 2, n = 5; exon 3, n = 3). Comparing the VAF kinetics between patients treated with and without dasatinib, baseline KIT mutations became subclonal (VAF <5%) in all patients receiving dasatinib (n = 8), whereas they were still detectable in 4/6 (67%) patients who were intensively treated without the addition of dasatinib. NRAS became subclonal (n = 8) irrespective of the treatment regimen. In one KIT mutated patient treated with dasatinib the baseline KIT Ex17D mutation (exon 17) was lost at the time of relapse, whereas the KIT D816V mutation (exon 8) was acquired instead. Gene set enrichment analyses revealed different mutation signatures at diagnosis and relapse: At diagnosis, there was a significant enrichment for genes associated with MYC overexpression. Variants that were recurrently present at diagnosis and relapse showed enrichment for genes affected in KRAS overexpression models. Relapse samples were additionally enriched for gene mutations involved in the mitotic spindle assembly.

Summary/Conclusions: Differences in the allelic composition were found between diagnosis and relapse regardless of the CBP-AML subtype. Our data suggest that the KIT clone might be successfully eradicated under dasatinib treatment whereas persistence of KIT mutant clones was more commonly seen under conventional chemotherapy. The frequent loss of KIT and NRAS mutations during therapy suggests that relapse is triggered by alternative genetic lesions. Relapsed disease may represent a distinct biology which is characterized by mutations that cluster in different pathways. Further analyses are ongoing including study cohort expansion, as well as inclusion of RNA sequencing results.

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P38β 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 anticancer activity of PP2A activating drugs (PADs) depends on the interaction/sequestration of SET, pointing out the significance of this oncogene in AML. Drug inhibition of several MAPKs in AML cell lines showed that only p38 inhibitors activate PP2A and decrease SET protein.

Aims: Therefore, we hypothesized that p38 could regulate SET at posttranslational level, leading to PP2A inactivation.

Methods: AML cell lines and primary human samples were analyzed by western blot, immunoprecipitation, immunofluorescence, treatment with pharmacological inhibitors and siRNAs. Phosphorylation assays by in vitro kinase assay with recombinant proteins were performed.

Results: Knockdown of the two major isoforms of p38-MAPK, p38α and p38β, demonstrated that only p38β was able to reduce SET protein levels and increase PP2A activity. To decipher this mechanism of action, we performed protein immunoprecipitation and immunofluorescence in the AML cell lines HL-60 and MOLM-13. p38β co-localized and bound to SET mostly in the cytoplasm stabilizing it, since treatment with 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 but not expression of p38β protein levels than p38α, as well as 5 out of 7 AML primary patient samples. Furthermore, expression analysis in a large series of adult de novo AML cases previously reported (Cancer Genome Atlas Research Network, 2013) showed a positive correlation between p38β (MAPK11) and SET (R2=0.416, p<0.001), but not between p38α and SET. We and others have shown that PADs retain SET in the nucleus. Our results showed that p38 phosphorylates SET not directly, but through the activation of casein kinase 2 (CK2), leading to the retention of SET in the nucleus and, therefore, contributing to the inactivation of PP2A in AML cells. Of note, CK2 is overexpressed in both AML cell lines and patient samples.

Summary/Conclusions: p38 is able to activate CK2 which phosphorylates SET and, as consequence, facilities its trafficking to the cytoplasm, contributing to PP2A inactivation in AML cells. Moreover, p38β binds to SET in the cytoplasm, contributing to its stability and leading to PP2A inactivation. In this regard, we have preliminary evidences that combination therapy with PADs and the CK2 inhibitor CX4945 reduces significantly the viability of AML cells, supporting that novel treatment modalities that can target multiple components of the same pathway may help to achieve a more sustained therapeutic benefit.

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GENETIC LANDSCAPE OF ACUTE ERYTHROID LEUKEMIA


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Acute myeloid leukemia - Biology 4
Background: Acute erythroid leukemia (AEL) is a unique subtype of acute myeloid leukemia (AML) characterized by the predominance of erythroid components with increased ring sideroblasts as well as frequent myelodyplasia. However, due to its rarity, the molecular pathogenesis of AEL has not fully been elucidated, except for frequent TP53 mutations.

Aims: This study was designed to clarify the mutation profile of AEL distinct from other myeloid malignancies using targeted-sequencing.

Methods: We performed a comprehensive genetic study, in which paired tumor/normal DNA from 22 AEL cases were analyzed using whole exome sequencing (WES). Whole-exome sequencing data from 3 AELs generated by The Cancer Genome Atlas (TCGA) was also included in the analysis. Subsequently, a total of 84 AEL cases were screened for mutations in 67 driver genes associated with myeloid malignancies using targeted-capture sequencing, in which RNA bait 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 in other acute 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 (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 common targets of mutations, including SRSF2 (12%), U2AF1 (4.8%), WT1 (15%), TET2 (19%) and IDH1/2 (12%). TP53 mutations were mutually exclusive with cohesin mutations (p<0.01) and those in splicing machinery (p<0.01). Mutations of AML associated with mutational signatures of MLL-PTD were normally less frequently observed in de novo AML than in secondary AML.

Summary/Conclusions: TP53 mutations in AEL are frequent and frequently combined with cohesin mutations. The splicing machinery (18%) and epigenetic regulators (45%) were also common targets of mutations. The impact of mutated/altered cohesin complex function within the cohesin complex provides evidence of the impact of mutant TP53 function within the cohesin complex.

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THE MOLECULAR LANDSCAPE OF MLL-PTD AML: SPECIFIC CONCURRRENT MUTATIONS, CLINICAL OUTCOME AND GENE EXPRESSION SIGNATURES

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Background: Partial tandem duplications (PTDs) in the Mixed Lineage Leukemia (MLL) gene, currently known as Lysine Methyltransferase 2A (KMT2A) are acquired-in-frame internal duplications present in 5–11% of acute myeloid leukemia (AML). MLL-PTDs are predominantly present in cytogenetic normal AML and occasionally in AML with trisomy of chromosome 11. MLL-PTD may be present as a poor prognostic marker in AML.

Aims: Evaluate the mutational landscape, prognostic value and gene expression signatures of MLL-PTD AMLs in comparison to a well-characterized AML cohort without MLL-PTD.

Methods: cDNA of 2310 AML patients enrolled in the adult HOVON-SAKK clinical trials (from 1995 to 2013) were analyzed for the presence of an MLL-PTD. Mutational screening based on next generation sequencing (NGS) was performed using the Illumina TruSight Myeloid panel on the Illumina HiSeq2500. An independent cohort of 632 de novo AML patients without MLL-PTD served as control. The gene expression profiling was assessed of all AML cases using Affymetrix HumanU133 Plus2.0 GeneChips as previously described (Verhaak et al, 2010).

Results: MLL-PTD was detected in 118 (5.1%) out of 2310 AML patients. MLL-PTDs were significantly associated with trisomy 11: 7% vs 1% (p=0.0037), normal karyotype: 65% vs 53% (p=0.0102) and complex karyotype: 1% vs 14% (p=0.001). MLL-PTD was also a poor prognostic marker in the cohesin leukemia fusion genes. The targeted NGS was performed on 87 out of 118 patients due to unavailability of gDNA. The number of mutations detected in MLL-PTD AMLs ranged from 0–6 mutations with an average of 3 mutations per patient. The most frequently mutated genes in MLL-PTD AMLs were DNMT3A, FLT3-ITD, U2AF1, IDH2, and TET2. In the context of the 632 AMLs without MLL-PTD mutations in several genes appeared to be significantly associated with the MLL-PTD, i.e., FLT3-ITD: 34% vs 13% (p<0.0001), IDH2: 16% vs 9% (p=0.0133), U2AF1: 9% vs 3% (p=0.0158) and IDH2: 23% vs 12% (p=0.018) or inversely associated, i.e., NPM1: 3% vs 32% (p=0.0001), NPM1: 5% vs 22% (p=0.0002) and TP53: 3% vs 10% (p=0.0487). Overall, the numbers of mutations in the spliceosome (p=0.03), tumor suppressor (p=0.0388), and transcription factor genes (p=0.0408) were significantly higher in MLL-PTD AMLs compared to MLL wild-type AMLs. As expected, the MLL-PTD appeared to be significantly associated with inferior outcome (MLL-PTD (n=74) and without MLL-PTD (n=1764); OS: p=0.05). Association of the presence of an MLL-PTD with EFS was only borderline significant (p=0.07). Within MLL-PTD AML, DNMT3A mutations are associated with inferior overall survival (HR: 2.06; 95%CI: 1.19-3.58; p=0.010). Although low numbers, MLL-PTD AML patients that harbor NPM1 mutations do even worse (HR: 6.54; 95%CI: 2.45-17.49; p<0.001. In multivariate analysis both markers remain significant when compared with WBC counts and cytogenetics. Multiple homeobox-related gene family members were overexpressed in MLL-PTD AML. The top-35 differentially expressed genes included HOXB5, HOXB6, HOXB7, HOXB8, HOXB9 and NXK2.3. In an association model, which takes all other known subsets of AML into account, other HOX-related genes, such as HOXAT, HOXAX and NXK2.5, were also found to be significantly overexpressed in MLL-PTD AML. In contrast, these specific gene expression changes were absent in AML with translocations involving MLL on 11q23. Additional analyses in AML subsets based on the current mutations will be carried out to investigate whether these are limited to a subset of MLL-PTD AML molecular subsets.

Summary/Conclusions: MLL-PTD AML carries specific gene expression signatures and specific subsets of concurrent mutations with clinical value.

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EXPLORING THE IMPACT OF LOSS OF FUNCTION STAG2 MUTATIONS ON CHROMATIN ARCHITECTURE IN AML

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Background: The Cohesin complex is an evolutionarily conserved multimeric protein complex involved in DNA replication, DNA damage response, homologous recombination and long-range interaction between cis regulatory elements of the genome. Within hematological malignancies, alterations of Cohesin complex function is associated with a worse outcome in chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS) or Myeloproliferative neoplasm (MPN). Stag2 mutations have been associated with the occurrence of both MDS and MPN and has been linked to a worse clinical outcome in CML. In AML, STAG2 mutations have not been associated with a worse clinical outcome. Aims: To explore the impact of a loss of function STAG2 mutation on the chromatin architecture within a isogenic cell based model.

Methods: Using a CRISPR generated isogenic model we have investigated the impact of STAG2 loss on the chromatin architecture of a hematopoietic environment. Genome wide binding profiles for STAG2, STAG2, and CTFC were generated using ChiP-Seq to elucidate areas of differential between STAG2 carriers. 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 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 loss of function of STAG2, with an increase in binding peaks from ~17,000 to 25,000, however several sites identified by ChiP-Seq are not compensated for. Histone mark profiling identified 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 a loss of boundaries within the DNA damage response, homologous recombination and long-range interaction between cis regulatory elements of the genome. Within hematological malignancies, alterations of STAG2 mutations have been associated with a worse clinical outcome in chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS) or Myeloproliferative neoplasm (MPN).
NEXT GENERATION SEQUENCING TECHNIQUES REVEAL MOLECULAR MECHANISMS OF MYB REGULATION AND FUNCTION IN MLL-AF9 LEUKAEMIA

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Background: Mutations involving the MYB gene at 11q23 are found in 10% of adult and 18% of childhood acute myeloid leukaemia (AML) cases. The most frequently occurring ML1 mutations are chromosome translocations that fuse the MYB gene in-frame with a second partner gene, creating novel fusion proteins (M-LLFs). MLL-AF9 is the most common MLL-FP in AML. Despite much progress in the overall management of AML, patients carrying MLL-rearrangements still have a poor survival prognosis and limited response to existing therapy. This is in part due to the low therapeutic indices and narrow therapeutic windows of current chemotherapeutic agents, therefore underscoring the need to develop improved, targeted therapies. MYB is a direct downstream target of MLL-AF9. Recent studies indicate that MLL-AF9 leukemia cells are more affected by MYB knockdown compared to normal hematopoietic stem progenitor cells. This is despite the fact that MYB is known to be essential for the establishment of definitive hematopoiesis. This suggests that a therapeutic window may be achieved through targeting MYB. Therefore, by understanding more about the role of MYB in MLL-AF9 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 MYB, MLL-AF9, H3K27ac, H3K4me3 and H3K27me3 chromatin immunoprecipitation (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 leukemia, suggesting a molecular rational for using BCL2 inhibitors in MLL-AF9 leukaemia therapy.

CD123-SPECIFIC CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) is a heterogeneous disease characterized by clonal evolution of myeloid precursors in bone marrow and peripheral blood resulting in accumulation of leukemic blasts and severe impairment of normal haematopoiesis. Despite advances in our understanding of AML biology, development of novel therapies has been limited with 43% relapse rate and 19% patients never attaining clinical remission (CR) with frontline induction treatment. Chimeric antigen receptor (CARs) T cells specific for tumour-associated antigens are emerging to be an effective form of immunotherapy for AML. A small number of in vitro and in vivo studies have evaluated the efficacy and specificity of CAR T cell immunotherapy in AML by targeting interleukin three receptor alpha (IL3RA, CD123), a molecule over expressed on AML blasts and leukaemia stem cells (LSC) compared to normal haematopoietic stem cells (HSCs).

Aims: In this study, we investigated the efficacy of a second generation CAR expressing an scFv for a single-chain variable fragments (scFv) with different affinities for CD123 and evaluated the cytotoxic effect of different co-stimulatory domains (CD28 versus 41BB) using a co-culture assay. Furthermore, we also evaluated the cytotoxic effects of a dual targeting CAR (against CD123 and CD33) using the same assay conditions.

Methods: Six lentiviral constructs (two high, two moderate & two low affinity) were transduced (MOI 1:5) into peripheral blood mononuclear cells (PBMCs) from healthy donors and their cytotoxicity was examined by flow cytometry on leukemic cell lines; KG1 (CD123+, CD34+, CD33+), Kasumi-1 (CD123+, CD34+, CD33+), U937 (CD123+, CD34+, CD33+), K562 (CD123+, CD34+, CD33+) and AML mononuclear cells (MNCs).

Results: Flowcytometric analysis confirmed the expansion of T cells from PBMCs and the cytotoxicity of the six CARCD123 constructs against CD123+ve cells. The high affinity CARCD123 (4nM kD & 4nM kD K136Q) T cells demonstrated enhanced cytotoxicity compared to moderate (56nM kD, 56nM kD A105G) and low affinity (10 nM kD, 10 nM kD V24G) CARCD123 in both leukemic cell lines and also in allogenic AML MNCs. Both the highest affinity CARCD123 constructs were also tested in cell lines using increasing effector: target ratios (1:2, 1:4 & 1:10) displaying consistent cytotoxicity and were also effective against autologous AML MNCs (target cells) and PBMCs (effector cells) from two patients. T cell activation was confirmed by ELISA and showed increased IFN-γ (500-2000 fold) and TNF-α (150-200 fold) levels. Previous studies have confirmed the distinction in CAR efficiency using CD28 versus 41BB co-stimulatory domains; CD28 co-stimulation augmented, whereas 4-1BB co-stimulation reduced T cell exhaustion induced by continuous CAR signaling. To confirm persistence of the CAR cytotoxicity, we constructed a high affinity CAR substituting CD28 with a 4-1BB co-stimulatory domain and obtained similar cytotoxicity results on K562 and U937 cell lines. Furthermore, a novel dual targeting CAR in which the activation domain (CD3ζ) is directed against CD33 and the costimulatory domain (CD28) directed against CD123 enhanced the specificity of the CAR towards leukemic cells; reducing “on-target but off-organ effects”. Results obtained in co-culture assay against KG1 and K562 cell lines with varying effector: target ratios were demonstrated results similar to the high affinity single targeting CAR.

Figure 1.

Summary/Conclusions: 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.

TARGETED COMBINATION THERAPY WITH CDK4/6 INHIBITOR PALBOCICLIB IN AML

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Background: Acute myeloid leukemia (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 involves 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.
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We have recently discovered that FLT3-ITD+ AML cells are highly sensitive to the FDA-approved, forbidden CDK4/6 kinase inhibitor ribociclib (BYL719, Pfizer). The effect is ascribed to the transcriptional activity of CDK6 on FLT3 and PIM1 - a feature not shared by CDK4.

Aims: FLT3-TKI treatment provides short-term disease control but relapse invariably occurs within months. Acquired resistance on FLT3-ITD patients to primary FLT3-ITD patients and to xenograft models, where palbociclib treatment effectively repressed FLT3-ITD583Y 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 inhibits both AURK and AKT in mutant FLT3 cells, 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-ITD+ AML. Inhibitory effects are cell cycle specific as well as 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.

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CANNABINOIDS DERIVATIVES MODIFY THE PATTERN OF SPHINGOLIPIDS IN ACRUTE 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 signalling 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 cannabinoid 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 small molecules and MS/MS were used. To assess the anti-leukemia effect of cannabinoids we analyzed cell viability by MTT and flow cytometry using six small molecules and MS/MS were used. To assess the anti-tumor effect of cannabinoids on AML cell lines, we used a cell proliferation 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-ITD583Y AML biopsies.

Results: Palbociclib impaired the viability of murine Ba/F3 cells with FLT3-ITD (80% inhibition at 1μM). The effect was not observed in primary FLT3-ITD patient samples and to xenograft models, where palbociclib treatment effectively repressed FLT3-ITD583Y 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 inhibits both AURK and AKT in mutant Ba/F3 cells, 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-ITD+ AML. Inhibitory effects are cell cycle specific as well as 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.

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PROFILING THE MUTATIONAL LANDSCAPE OF ACUTE MYELOID LEUKEMIA AT RELAPSE AFTER CHEMOTHERAPY AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPANTATION

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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=30). Sequencing was performed on an Illumina HiSeq2500 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 0-8), 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.

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Abstract withdrawn.

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**AML PATIENTS AGED ≥75 YEARS ENROLLED INTO AMLCG TRIALS: DO GENETIC ALTERATIONS IMPACT CLINICAL OUTCOME IN VERY OLD, INTENSIVELY TREATED PATIENTS?**


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**Background:** Acute myeloid leukemia (AML) is a disease of the elderly (median age at diagnosis ~68 years). The prognosis of elderly patients (pts) is poor. Advanced age often leads to the judgement that pts are unfit for induction chemotherapy, although several trials have revealed a positive impact of intensive induction therapy in terms of sustained remissions and long-term survival in a subset of elderly pts.

**Aims:** We sought to validate existing risk classification systems and identify genetic factors associated with clinical outcomes in very old AML pts who received induction chemotherapy.

**Methods:** We identified 151 AML pts aged ≥75 years who received intensive induction therapy in the AMLCG-1999 trial with suitable material for genetic
analyses. 81% of pts had de novo AML, 15% secondary AML, 3% therapy-related AML and 2% high-risk MDS. Recurrent gene mutations in AML were studied from bone marrow aspirates or peripheral blood using a targeted leukemia genotyping assay covering 68 genes. We analyzed known mutational hotspots or the entire coding sequence of the genes by multiplexed amplicon sequencing (Agilent Technologies, mean target coverage of 460x). We studied association of gene mutations with other potential prognostic factors which might influence the clinical outcome.

Results: The median age in the total cohort was 76 years (range: 75-86). 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 23%. According to the ELN 2017 classification, 21% 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=0.02). In the adverse group, pts with the 13 IDs mutated (p<.001; Figure). Moreover, 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 leukemia-associated 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=0.09; EZH2: p=0.05). FLT3-ITD mutations were identified in 29 pts (19%), but had no impact on OS (p=.297). The NPM1 and/or FLT3-ITD genetic genotype also did not associate with OS. Notably, none of the 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 wild-type pts (p<.001; Figure). The positive impact of mutated NPM1 on OS was restricted to pts with IDH1 wild-type 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 the incidence of AML increases with age, advanced age and comorbidities may preclude the administration of intensive therapy altogether.

ACP-1726 (Astarbine) is a novel compound of cytarabine covalently bound to asparagine. It acts as a pro-drug of cytarabine, enabling delivery of high cytarabine doses to target cells with lower systemic exposure to the free drug and relative sparing of normal tissues. As such, BST-236 may serve as an ideal therapy for leukemia, particularly for delivering high doses of cytarabine to target the unfit or older patients. 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/day), each composed of 3-6 patients. Treatment was administered in 1-hour daily infusions 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. Among 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) with up to 5 months follow-up of the CR patients was 11 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 higher 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, 210 | haematologica | 2017; 102(s2)
Background: Mixed phenotype acute leukemia (MPAL) is a rare subgroup of acute leukemia characterized by blasts that show immunophenotypicss of both myeloid and lymphoid lineages and therefore not traceable to single lineage of origin. Diagnosis of MPAL is challenging due to the possible discrepancy between immunophenotype and morphology. Clinically, MPAL has poor prognosis and poses therapeutic challenges. Genetic basis of MPAL is not well understood.

Aims: To clarify the underlying pathogenesis of MPAL and provide clue on future personalized therapy in MPAL, we performed comprehensive molecular characterization of adult MPAL.

Methods: We studied 31 patients with adult MPAL (median age 53) that met 2008 FAB criteria for diagnosis and treatment. Myelo-lymphoblastic transformations were studied by targeted capture exonese 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. Of the 45 cases of which pre-treatment samples were sequenced and aligned together with the mixed immunophenotypic features, MPAL had both AML-type and ALL-type mutations. However, NPM1 mutation was specific to AML and was not found in MPAL cases. Myeloid-T and myeloid-B showed distinct patterns of somatic mutations. Genes in which mutations were enriched in myeloid-T than in AML included NPM1, BIRC7, NUP98-ING3, NOTCH1, and TET2. In cases with suspected NPM1 fusion, NPM1 transcript with fusion partner was confirmed in 7/10 cases. In 42/45 (93%) cases with a recurrent rearrangement identified by RNA sequencing, the respective fusion transcript was confirmed in 41/45 (91%). Genes that are essential in T-cell receptor (TCR) signaling (CD3D, CD7, CD247, LCK, PRKRC, CCR9, and TCLA1) were differentially methylated and consequently differentially expressed between myeloid-T and myeloid-B. Copy number analysis showed that 1/11 cases with novel translocations had amplification of one of the partner genes, was amplified and was overexpressed. RNA sequencing revealed known translocations such as NUP98-NUP98 and KMT2A-MALT4, in addition to the novel translocations such as FOXP1-DNAJC15, FLI1-IFT46, and ITPRB-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, CpG methylation, and gene expression differences. Therapy for MPAL may need to be personalized based on genomic profiles.
men (standard group). Re-induction and consolidation therapy was performed according to a consistent strategy and post-remission therapy was mainly based on hematopoietic cell transplantation.

**Results:** Early intensified group was consisted of younger patients (median age, 37 years old [range 17-69]) vs 45 years in 3+7 vs 43 years in 3+10 subgroup) and larger proportion of t(8;21) (n=102 [27.7%] vs 3+7 [n=33, 7.1%] vs 3+10 [n=78, 12.6%], P<0.001). Also, initial BM blast cell counts were higher in two intensified groups (73.3% in 3+5 and 70.1% in 3+10) compared to 3+7 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+5 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 relapses were lowest in patients younger than 55 years (4% vs 11%, P=0.002) and favorable to intermediate-risk group (9.9% vs 20.2%, P<0.001) after intensified induction. In young patients with favorable to intermediate-risk karyotype, intensified group showed superior 5-year OS (55.0% vs 45.5%, P=0.010) and lower long-term relapse rate (32.2% vs 38.0%, P=0.084), but multivariate analysis revealed no effects for both OS and CR. In patients older than 55 years, intensified group showed inferior 5-year OS (19.2% vs 22.8%, P=0.014) with higher early death rate (17.6% vs 6.3%, P=0.015), and multivariate analysis also showed intensified induction was related inferior OS (HR=1.89, 95%CI; 1.14-3.15, P=0.013).

**Summary/Conclusions:** Our data revealed that intensified induction chemotherapy was not influential for poor-risk karyotype, while higher post-induction CR rate and low pre-HCT relapse was shown in young patients with favorable to intermediate-risk karyotype although it was not influential for final OS and CR rate. In elderly patients, intensified induction chemotherapy was related with higher early death rate which finally showed poor OS.

**P552**

**VARIANT FLT3 MUTATIONS CAN BE ERADICATED BY CYTARABINE/ANTHRACYCLINE/CRENOLANIB INDUCTION IN ADULT PATIENTS WITH NEWLY DIAGNOSED FLT3 (ITD/TKD) MUTANT AML**

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**Background:** Patients (pts) with FLT3-internal tandem duplication (ITD) and FLT3-D835 mutant AML have a high relapse rate. These relapses are typically due to outgrowth of mutant 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 add to the typical FLT3-ITD, as well as 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/crenolanib induction followed by high dose cytarabine (HiDAC) consolidation. Crenolanib 100mg TID was started on day 9 of induction and continued till next chemotherapy. Crenolanib was administered following consolidation and allogeneic stem cell transplantation. Bone marrow samples were collected at baseline and at the time of remission assessment. Sequencing of the entire FLT3 gene was performed through FoundationOne Heme panel (n=18) and MSKCC multigene panel (n=5). Sequencing of exons 14, 15, 16, and 20 was performed through the Rapid Heme Panel at Dana-Farber Cancer Institute in additional 6 pts.

**Results:** 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 N841IT/T (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 pts), 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/crenolanib induction). The pt with FLT3-D835Yand N841IT achieved a CR after cytarabine/anthracycline/crenolanib induction and one cycle of HiDAC consolidation. All pts received FLT3-v and have remained FLT3-ve out of 4 pts received 1-4 cycles of HiDAC consolidation followed by crenolanib maintenance. Only one pt underwent allo SCT. With a median follow up of 13 months, one pt relapsed (at 8.4-month following treatment). This 61F pt was found to have FLT3-ITD, D593H and i836del FLT3 abnormalities at the time of diagnosis. A full FoundationOne gene panel done at the time of relapse, showed no residual FLT3 mutant clones.

**Summary/Conclusions:** This abstract reports multiple novel variant FLT3 mutations in adult pts with newly diagnosed FLT3-ITD or FLT3-D835 mutant AML. The allelic burden of these FLT3 variant mutations can sometime be higher than that of FLT3-ITD. Detailed FLT3 analyses in this subset of pts suggests that crenolanib in combination with standard induction chemotherapy has the ability to eradicate variant FLT3 clones. All 4 pts treated with chemotherapy followed by crenolanib showed clearance of FLT3-ITD, TKD, as well as other novel variants. To achieve maximal clinical benefit, a potent pan-FLT3 inhibitor with the ability to inhibit IDT, D835, as well as other activating mutations maybe beneficial.

**P553**

**PATIENTS WITH ACUTE MYELOID LEUKEMIA WHO HAVE MUTATIONS IN IDH1 OR IDH2 RESPOND WELL TO INDUCTION CHEMOTHERAPY WITH “7+3” DESPITE THE PRESENCE OF COMPLEX KARYOTYPE OR FLT3-ITD**

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**Background:** Mutations in isocitrate dehydrogenase isoforms 1 and 2 (IDH1/IDH2) occur in 8-12% of patients with acute myeloid leukemia (AML). Mutant IDH enzymes catalyze the conversion of alpha ketoglutarate to beta hydroxyglutarate. Increased concentrations of intracellular 2-HG lead to histone hypermethylation and a block in cellular differentiation and may also lead to suppression of homologous recombination. Previous studies of outcomes in patients with FLT3-ITD have suggested that the combination of FLT3 mutations in induction regimens. 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 R1400 mutations. Co-occurring mutations in FL3T (ITD or TKD), DNMT3A or NRAS were not predictive of responses to induction chemotherapy.

Summary/Conclusions: Induction chemotherapy with 7+3 leads to a robust CR rate of 82% in patients with AML that harbor and IDH1 or IDH2 mutation. CR is not affected by IDH2 isoform (R172 or R140), although more patients with IDH2 mutations required two cycles of chemotherapy to achieve a remission. Karyotypic abnormalities did not influence the response to induction chemotherapy, nor did the presence of co-occurring FLT3-ITD, FLT3-TKD or IDH mutations. Co-occurring mutations in FLT3 (ITD or TKD), DNMT3A or NRAS were not predictive of responses to induction chemotherapy.
However, the correlation is only valid for CYT-IDA while the PM Test was applied in 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 chemotherapeutic 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 AZA CIDINE AND INDUCTION CHEMOTHERAPY IN PATIENTS >60 YEARS OLD WITH NEWLY DIAGNOSED AML ELIGIBLE FOR CHEMOTHERAPY: RESULTS OF THE DRKS00004519 STUDY OF THE EAST GERMAN STUDY GROUP
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Background: AML treatment in elderly patients (pts) >60 years (y) with intensive chemotherapy (IC) or azacitidine (AZA) are not necessarily mutually exclusive. Aims: Results of the multicenter DRKS00004519 (RAS-AZIC) study of the East German Study Group (OSHO) which evaluated first-line treatment with AZA 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 d 1-3 and cytarabine 1g/m2/BID on d 1, 3, 5, 7) on d17 was established through a 3+3 design. In the multicenter phase II part (figure), upfront AZA was sequentially followed by AZA or IC based on d15 bone marrow (BM) blasts (<45 vs >45%) and CR/CRi on d56 which were both previously identified as early predictors for long-term response to AZA in AML (Al-Ali et al. Leuk Lymph 2014). The primary endpoint was response rate (CR/CRi, CR) and mortality 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 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.

Results: Median age was 70y (52% males), de novo AML was present in 65% of pts. Median BM blasts and WBC were 50% and 4.4x109/L respectively. Genetic risk was high in 30%, intermediate in 55%, and favorable in 15%. FLT3 and NPM1 were mutated in 12% and 22% respectively. All pts received first-line AZA. Only lower baseline blasts correlated with 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 (54.5% continued with AZA; 46.5% received IC). Of 152 AZA cycles given till d56, 33.6% were applied in an outpatient setting. Until d90, one IC cycle was needed in 77 (68.8%) pts. In the intention-to-treat cohort (n=112), 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/FLT3wt (p=0.003). Protocol therapy was generally well tolerated. Constipation grade 1-2 was the most frequently reported AE under AZA (48%). The most frequent grade 3+4 non-hematologic AE was infection (IC [47%]; AZA [20%]).

Figure 1.
Summary/Conclusions: 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 recovery (CRp) could receive up to 2 cycles of consolidation therapy. Note, the study was not powered for this subgroup analysis.

Results: A total of 304 patients were enrolled and received study treatment, including 62 (20%) patients with tAML (CPX-351 arm, n=30; 7+3 arm, n=32). Characteristics of tAML patients were similar between the CPX-351 and 7+3 arms: median age was 69.0 versus 67.5 years, and 47% versus 53% were male. Prior treatment in patients with tAML included prior non-anthracycline chemotherapy alone (26%), radiation alone (26%), non-anthracycline chemotherapy + radiation (32%), non-anthracycline + anthracycline chemotherapy (5%), and non-anthracycline + anthracycline chemotherapy + radiation (11%). CPX-351 was associated with a significant OS benefit versus 7+3 in older tAML patients and numerically longer event-free survival and remission duration (Figure). Additionally, a greater proportion of tAML patients in the CPX-351 arm versus the 7+3 arm achieved CR+CRi (47% vs 38%, respectively; odds ratio=1.33 [95% CI: 0.47, 3.81]) and proceeded to stem cell transplantation (37% vs 27%; odds ratio=1.54 [95% CI: 0.53, 4.49]). Serious treatment-emergent adverse events (TEAEs) were reported for 18/30 (60%) of tAML patients in the CPX-351 arm and 12/32 (38%) of tAML patients in the 7+3 arm; the observed difference in serious TEAEs in this subpopulation appeared to primarily be due to the incidence of febrile neutropenia (n=6/30 [20%] versus 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 ferritin have been correlated to a shorter overall survival (OS) and an increased relapse risk. We have previously shown that hyperferritinemia at diagnosis has a strong prognostic impact in a cohort of 162 AML patients with intermediate cytogenetic risk and younger than 60.

Aims: We now extend the analysis to all age and cytogenetic risk, in order to confirm the impact of hyperferritinemia in AML.

Methods: This study included 525 adult AML patients (excluding acute 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 10.0×109/L (IQR, 2.5-41.5). Cytogenetic risk was favorable, intermediate and adverse in 9.2% (N=48), 71.8% (N=374) and 19% (N=99) respectively; ELN classification was favorable, intermediate-I, intermediate-II, adverse and unknown in 21.0% (N=110), 25.5% (N=134), 22.3% (N=117), 18.9% (N=99) and 12.4% (N=65) respectively. Median ferritinemia at AML diagnosis was 715 µg/L (IQR, 372-1304), ranging from 34µg/L to 70759 µg/L (upper normal limit [UNL]; 300µg/L). 421 patients achieved complete remission (CR; 80.2%). Early death and treatment failure rates were 7.8% (N=41) and 12% (N=63) respectively. 169 patients underwent allogeneic HSCT in first CR (32.2%). Median DFS was 19.8 months (IQR, 8.4-Not Reached). Ferritinemia had a significant impact on DFS: median DFS was 21.2 months in patients with ferritinemia ≤2100 µg/L (7-fold UNL), and 17.7 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=0.0011; ≥7-fold UNL, p=0.0004; ≥3-fold UNL, p<0.0001 and ≥3-fold UNL, p<0.0001 respectively).

Figure 1.

Summary/Conclusions: In conclusion, hyperferritinemia is a prognostic marker independent from well-acknowledged factors, such as cytogenetics and molecular abnormalities. Ferritinemia should be included at AML diagnosis workup as it provides reproducible information on short and long-term outcome for AML patients of any subgroup. The putative link between hyperferritinemia, inflammation and chemoresistance should be investigated.

P558

NGS ANALYSIS OF 474 BONE MARROW SAMPLES FROM 157 AML PATIENTS TREATED WITH AZACITIDINE–IMPACT OF AGE ON MUTATIONAL LOAD

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Background: Recent publications have shown the prognostic value of performing next generation sequencing (NGS) analyses in patients (pts) with acute myeloid leukemia (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 haematologica | 2017; 102(s2) | 215

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the number of distinct mutations detected in 157 AML pts treated with azacitidine and receiving ≥3 cycles of therapy. We now extend the analysis to all age and cytogenetic risk, in order to confirm the impact of hyperferritinemia in AML.
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 Hematological Agents from two centers (Salzburg, Wels-Grieskirchen) using a 53-gene panel (all exons). NGS was performed by Qiagen. Minimum coverage: 1500x. All mutations were checked against COSMIC-v79, ClinVar, ICGC, DocCM, dbsnip and Varsome databases. For comparison of categorical variables Chi-squared test was used, for comparison of medians Student’s T-test was used.

Results: The rate of secondary (s)AML was significantly lower in pts <75 (n=85), vs ≥75 years (n=54) (66.0 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 vs ≥75 years (10.2 vs 8.6 mutated genes/patient; P=0.030 and 12.9 vs 10.5 mutations/patient; 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 patients had more than one marrow sample with NGS results. Analysis of paired samples revealed that mutational load was significantly higher during/post-AZA vs before AZA in both age groups (Figure 1C-D). In total, 60.4%, 15.8%, 8.6%, 3.6% and 11.5% of pts acquired 0, 1, 2, 3, 4-13 additional mutations, respectively. No relevant differences between pts < vs ≥75 years were found (data not shown). When comparing the delta of mutations before vs during/after AZA according to age group, no significant difference was found (Figure 1E).

Table 1.

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 & Vassilou, Dis Model Mech 2014). It seems however, that a higher mutational load (average 3-14 mutations) per pt can be found using whole genome/whole exome sequencing (The Cancer Genome Atlas Research Network, NEJM 2013; Merlevede et al, Nat Commun 2016). We hypothesize that the higher observed number of mutations in our study may be due to the high coverage (minimum 1500x) we used (most previous publications had a median/average coverage of 500x). While age <75 years seems to coincide with a higher mutational load both before AZA start and during/after AZA in our cohort, it does not seem to predispose to the acquisition of more mutations during/after AZA. Higher mutational load in AML pts <75 years did not go hand in hand with a higher rate of known/presumed adverse prognostic baseline factors such as adverse cytogenetics, monosomal karyotype, or sAML at AZA start. We thus hypothesize that the biology of the disease may generally be more aggressive in younger pts. Correlation analyses of age and mutational load with response and survival will be in our final presentation.
AML showed higher therapy-related mortality (TRM) rate. However, multivariate rate and inferior survival outcome compared to normocellular AML, and hypotreated group (n=1386), hypo-AML and AML-MRC both showed higher relapse survival outcome compared to normocellular

Results: Signal rate was assessed in 152 patients with FLT3-ITD and -TKD mutations who had received ≥80mg gilteritinib. Of these patients, 136 had FLT3-ITD mutations with or without concomitant TKD mutations, and 16 had FLT3-TKD mutations only. Median ITD and TKD signal ratios were 0.84 and 0.5, respectively. Patients with FLT3-ITD signal ratios that were above or below the median ITD signal ratio had OS durations of 216 and 213 days, respectively. No significant difference in median OS was observed between patients in the highest and lowest FLT3-ITD signal ratio quartiles (Figure 1). Patients with TKD signal ratios that were above the median value (0.5) had a median OS of 202 days; those with TKD signal ratios below the median value had a significantly shorter median OS of 33.5 days (P<0.004; 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.

Background:

Hypocellular acute myeloid leukemia (hypo-AML) and AML with myelodysplasia-related change (AML-MRC) accounts for small proportion of adult AML. As the characteristics and outcomes are not well recognized.

Aims: We tried to analyze these specific groups and compared to normocellular AML.

Methods: After exclusion of secondary AML, therapy-related AML, and AML M3, we retrospectively analyzed 1593 AML cases between 2002 and 2013. We found 101 (6.3%) patients with hypo-AML and 164 (10.3%) patients with de novo AML-MRC. Hypo-AML was diagnosed with blast counts ≥20% within hypocellular (<20%) bone marrow (BM) biopsy specimens and age-related correction was considered. De novo AML-MRC was defined with multilineage dysplasia ≥10% for each lineage with blast counts ≥20% without history of antecedent hematologic disease. Patients (n=20) with both AML-MRC and hypo-AML were distributed in AML-MRC group.

Results: At the time of diagnosis, patients with hypo-AML were older (p=0.001) and significantly presented lower leukocyte and PB/BM blast counts (p<0.001). Patients with AML-MRC were older and lower hemoglobin level with lower PB/BM blast counts (p=0.001) compared to normocellular de novo AML. In both groups, the risk of karyotype was poorer. In untreated group (n=207), hypo-AML showed longer survival outcome compared to normocellular de novo AML and AML-MRC. In treated group (n=1386), hypo-AML and AML-MRC both showed higher relapse rate and inferior survival outcome compared to normocellular AML, and hypo-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).

Figure 2.

Summary/Conclusions: The long-term outcome of hypo-aML and AML-MRC were poorer than normocellular de novo AML, mainly due to older age and large proportion of adverse-risk karyotype which caused unavailable condition for HCT.

P562

INITIAL RESULTS FROM A FIRST-IN-HUMAN STUDY OF IMGN779, A CD33-TARGETING ANTIBODY-DRUG CONJUGATE (ADC) WITH NOVEL DNA ALKYLATING ACTIVITY, IN PATIENTS WITH RELAPSED OR REFRactory AML

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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+AML, 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 followed a standard 3+3 design, began with a starting dose of 0.02mg/kg. IMGN779 was administered intravenously once every 2 weeks on days 1 and 15 as part of a 28-day cycle. Adverse events (AEs) were evaluated using NCI-CTC v4.03.

Results: As of February 2017, a total of 17 patients (9 female, 8 male) with a median age of 62 years have received IMGN779 treatment. Five dose levels have been completed, with escalation proceeding from 0.02–0.26mg/kg. AEs were as expected for this relapsed/refractory AML population including cytopenias and constitutional symptoms. No relationship between frequency or severity...
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.

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 on Days 1, 8, 15 of Cycle 1, Day 1 of Cycle 2-6, followed by Cycle 9 & 12. TGR-1202 was started at 800mg QD with a -1 dose reduction cohort at 600mg if not tolerated in ≥2/6 pts. Benda was dosed at 90mg/m² on Days 1 & 2 of Cycles 1-6 only. Primary endpoints included safety and efficacy (Cheson 2007).

Results: Twenty-three pts were evaluable for safety: 15 diffuse large B-cell (DLBCL) and 8 follicular (FL). Med age 68 yo (range 31-81); 12 M/11 F; median prior treatment regimens=2 (range 1-6); 12 pts (52%) were refractory to their immediate prior treatment and to prior CD20 therapy, and 7 patients had progressed post-transplant. ECOG PS 0/1/2 (3/18/2). Initially 2/4 pts at 800mg TGR-1202 experienced AEs in Cycle 1 that led to treatment interruption (rash, neutropenia) thus the 600mg dose of TGR-1202 was explored. No additional Cycle 1 treatment delays were reported at the 600mg dose level, which was later expanded and the 800mg TGR-1202 dose is now being evaluated with stricter eligibility criteria to require an ANC of ≥1.0, and the use of growth factor support in cycle 1 is now encouraged. The most common AE’s included diarrhea (35%; G3/4 5%), decreased appetite (35%; G3/4 4%), nausea (30%; G3/4 4%), asthenia (26%; G3/4 4%) and neutropenia (22%). The only Grade 3/4 AE reported in >10% of pts was neutropenia (22%). Two pts had a TGR-1202 dose reduction. Nineteen pts (11 DLBCL/8 FL) were evaluable for efficacy: ORR amongst all pts was 79% (15/19) with 42% (8/19) achieving a complete response (CR), of which 5 were DLBCL and 3 FL. ORR in the respective groups as follows:

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>11</td>
<td>55%</td>
<td>5 (45%)</td>
<td>3 (25%)</td>
<td>1 (9%)</td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>8</td>
<td>88%</td>
<td>5 (62%)</td>
<td>2 (25%)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Median follow-up time on study is 6 mos for all pts (range 1-14+ mos).

Summary/Conclusions: The combination of UTX, TGR-1202, and bendamustine has exhibited manageable toxicity with significant activity in advanced DLBCL and FL pts including an encouraging 42% CR rate (45% in DLBCL and 38% in FL). Enrollment continues at the 800mg TGR-1202 dose level with the use of growth factor prophylaxis. Safety and efficacy data for all pts will be updated at the meeting. Based upon the early activity of the triplet, future registration directed studies are being planned.

P564

VENETOCLAX (VEN) IN PATIENTS WITH RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA (NHL)


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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 300–1200mg. Adverse events (AEs) were assessed by NCI-CTCAE v4.0 and response by 2007 Chevalier de Igang response criteria, utilizing CT scans beginning at wk 6.

Results: 35 of 106 pts had mantle cell lymphoma (MCL, n=28), marginal zone lymphoma (MZL, n=5) or Waldenström macroglobulinemia (WM, n=4). Most common grade 3/4 treatment emergent AEs were nausea (51%), diarrhea (49%) and fatigue (34%); grade 3/4 AEs in >10% of pts were neutropenia and anemia (17% each). Laboratory TLS was reported in a single pt (bulky MCL). MCL pts (median age: 72 years) had received a median of 3 (1–7) prior treatments (tx). Median time from start of prior tx to start of VEN was 13 mo (2–148) and time on VEN was 16, 15, 74 mo and time on VEN was 42, 17, 54, 20 mo. All pts achieved PR (at wks 6 [n=10], 2 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 and other NHL. Further investigation of VEN in each disease is indicated.

P565

WHOLE BODY DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING IS A GOOD PREDICTOR OF TREATMENT OUTCOME AFTER ONE CYCLE OF IMMUNOCHEMOTHERAPY IN AGGRESSIVE LYMPHOMA


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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 effects and reduce exposure to ineffective drugs. Interim fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) after 2-4 cycles of immunotherapy has prognostic value shown for some lymphomas, but its role in treatment adaptation is still considered experimental. Disadvantages of this technique are the significant amount of false negative test results and due to a tumor-nodemeta-inflamed respiratory response as well as substantial patient radiation exposure.

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 (DLBCL), 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 (1 cycle immunotherapy), and end-of-treatment FDG-PET/CT. 38/46 had an interim FDG-PET/CT. Additional International prognostic index (IPI), immunohistochemical markers Ki-67, Bcl-2 and Bcl-2 were evaluated for their predictive value. WB-DWI/MRI were assessed quantitatively with diffusion tensor imaging (DTI), fractional anisotropy (FA), additivity baseline and intersec scan (Delta). Statistical analysis consisted of Kaplan-Meier survival analysis univariate and multivariate Cox regression analysis with disease-free-survival (DFS) as outcome measure.

Results: Median follow-up time was 43 months (4-70 months). Thirty-three patients achieved complete remission (CR), 4 progressed and remained on therapy, 5 had disease progression and 9 had recurrent disease. Patients were non-responders according to WB-DWI/MRI in case of an ADCmean decrease for lymphoid tissue or less than 10% b1000 mean decrease in bone or a b1000increase of less than 6% in extranodal lesions. WB-DWI/MRI predicted DFS correctly in 45/46 (96%) [p<0.001; hazard ratio (HR) 66.6, (CI 95% 8.5-523.2)]; end-of-treatment FDG-PET/CT was additive in 37/46 (80%) [p=0.004; HR 5.1, (CI 95% 1.7-15.4.)], and interim FDG-PET/CT was additive in 27/38 (71%) [p=0.042; HR 3.5, (CI 95% 1.0-11.5.)]. ORI score neither histological or immunohistochemical parameters demonstrated a significant predictive value. Multivariate analysis revealed WB-DWI/MRI as the only independent prognostic factor (p<0.001).

Summary/Conclusions: WB-DWI/MRI can accurately predict treatment outcome in aggressive NHL after only one cycle of immunotherapy (2-3 weeks) without the burden of radiation exposure.

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P567

PREVALENCE AND PROGNOSTIC VALUE OF MYD88 AND CD79B MUTATIONS IN IMMUNE-PRIVILEGED SITE AND (EXTRA)NODAL DLBCLS

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Background: Inbrutinib (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 further characterize the efficacy of IBR 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 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 3 (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).

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.

Table 1.
Background: Activating mutations in CD79B and MYD88 are important molecular drivers of a subset of diffuse large B-cell lymphomas (DLBCLs), activating the B-cell receptor and toll-like receptor pathways, respectively. Interestingly, the frequency of these mutations differs greatly among DLBCLs at different anatomical sites, with a remarkably high prevalence at immune-privileged (IP) sites (central nervous system and testis). Recent studies suggest that these mutations are associated with an unfavorable prognosis. However, the prognostic value in relation to the site of presentation has not yet been explored.

Aims: To investigate if mutations in MYD88 and CD79B are independent prognosticators for overall survival (OS) in DLBCL, particularly in patients with lymphomas at IP sites, for which a high prevalence of these mutations was reported.

Methods: In this retrospective study, we investigated a large clinically annotated cohort of 189 consecutive primary DLBCLs, including primarily nodal (N=64), primarily extranodal (N=74) and IP localizations (N=51). Patients were diagnosed between 1990-2015 at the Academic Medical Center, (University of Amsterdam) or other Dutch hospitals. The vast majority was treated with (R-)CHOP (N=143) or other immune-chemotherapies (N=16). Detailed clinical characteristics of all patients were collected. For all patients BCL2, BCL6, and MYC translocations, Epstein Bar Virus (EBV) status and the mutational status of MYD88 and CD79B were assessed, employing methods described previously (Kraan et al., BCJ 2013).

Results: Translocations in BCL2, BCL6 and MYC were identified in 14, 32 and 13 patients, respectively and 23 EBV-positive cases were found. MYD88 and 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=14). Patients with a MYD88 mutation demonstrated a significantly inferior 5-years OS compared to DLBCL with wild-type MYD88 (Log Rank test P=0.002, Figure-1A). This prognostic significance was also found for DLBCLs with IP sites (Figure-1B, LR P=0.029). Coexistence of a CD79B mutation did not impact the prognostic significance of MYD88. Multivariable Cox regression analysis, including clinical and molecular characteristics (i.e. age, translocations, EBV, CD79B, etc.) demonstrated that MYD88 mutations are an independent unfavorable prognostic factor for OS, in particular in DLBCL 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.

Summary/Conclusions: Our study demonstrates that mutated MYD88 is an independent unfavorable prognostic factor for OS, in particular in DLBCL 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.

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HIV-INFECTED PATIENTS WITH RELAPSED NON-HODGKIN LYMPHOMA (NHL) OR HODGKIN LYMPHOMA (HL): RESULTS FROM THE GERMAN HIV-RELATED LYMPHOMA COHORT STUDY

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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.

Aims: To analyze the outcome of pts with HIV-related lymphoma who experienced a relapse after having achieved a complete response to first line therapy.

Methods: This prospective multicenter cohort study includes adult HIV-1 infected pts with biopsy or cytoplogically 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 45.8 yrs (range, 22–74.7). 344 pts (89%) 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). As of June 2015, 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 6 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=14). 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.

Aims: The study investigated the prognostic significance of baseline NCCN-IPI and post-treatment PET-CT scan, assessed by Deauville score, in patients with nodal PTCL. The primary aim was to establish a risk model for nodal PTCL patients based on NCCN-IPI, a clinical tool, and post-treatment PET-CT scan indicating tumor viability.

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 using 5-point Deauville score. The study excluded ALCL-ALK+ due to well-known better survival.

Results: A total of 396 patients were screened for eligibility. Seventy patients were excluded from the analysis due to following reasons: unavailable pre- or post-treatment PET scans, no systemic treatment, uncertain histology, and post-treatment PET-CT scan indicating tumor viability.

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 1.129-2.288, P = 0.004; for post-treatment Deauville score 3, HR 3.647, 95% CI 2.384-5.557, P < 0.001). We stratified patients into 5 groups based on risk of progression: a low (NCCN-IPI and Deauville score 1-2), intermediate (NCCN-IPI and Deauville score 3), or high (NCCN-IPI and Deauville score 4-5). The risk model showed a strong association with PFS and OS (Figure 1).

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.
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 localization of signaling molecules to the inner cell membrane. CXCL12 is a chemokine that is essential for hematopoietic stem cell (HSC) homing to the bone marrow and lymphoid organs and for maintenance of HSCs and immune cell progenitors. CXCL12 is known to signal in part through HRAS, a signaling protein that is uniquely farnesylated. Tipifarnib has previously been shown to be well tolerated and to have a 41% response rate (7 responses out of 17 patients) in patients (pts) with T-cell Non-Hodgkin Lymphoma, including 4 objective responses in 8 pts with peripheral T-cell lymphoma (PTCL) (Witzig et al, 2011). Building on this prior experience, we report herein the preliminary efficacy, safety and biomarker data from our ongoing Phase 2 study in PTCL.

Aims: This Phase 2 study is a multi-institutional, single-arm, open-label, two-stage (11+7) study designed to determine the efficacy and safety of tipifarnib in pts with relapsed/refractory (R/R) PTCL.

Methods: Pts with R/R PTCL after prior cytotoxic systemic therapy, aged ≥ 18 years old, and with a performance status of 0-2 were eligible. Informed consent was obtained. The following subtypes of PTCL were eligible for enrollment: PTCL, not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), ALK-positive and -negative anaplastic large cell lymphoma (ALCL), hepatosplenic T-cell lymphoma, enteropathy-associated T-cell lymphoma (EATL), extranodal natural killer (NK)-T-cell lymphoma, nasal type and subcutaneous panniculitis-like T-cell lymphoma. The primary endpoint of the study is overall response rate. Secondary endpoints include safety and tolerability, duration of response (DOR) and progression free survival (PFS). Based on activity observed in the first 18 pts in the study, the protocol has been amended and enrollment is ongoing to an expansion cohort in AITL (N=12). Enrolled pts are treated with tipifarnib 600mg 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.

Results: At data cut-off (2/15/2017), 18 pts (2 AITL, 1 ALK- ALCL, 15 PTCL-NOS) were treated with tipifarnib. Most common treatment-related AE (grade ≥ 3) were myelosuppression, including neutropenia (61%), anemia (39%) and thrombocytopenia (39%). 3 pts achieved a partial response (2 PTCL; 1 AITL; 1 PTCL-NOS) and 3 additional pts experienced stable disease >6 months. Tumor DNA from 16 pts was sequenced using NGS tools. A high rate of CXCL12 3’UTR single nucleotide variation (SNV) was observed. Seven of 16 pts carried the rs2839695 variant while an additional patient carried a novel variant. The presence of 3’UTR SNVs was associated with low levels of CXCL12 gene expression and disease progression (Figure 1) 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 AITL histology, absence of 3’UTR CXCL12 SNV and expressed high levels of mRNA for this chemokine. Testing of circulating CXCL12 levels is ongoing.

P572

BAM CONDITIONING BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA: A RETROSPECTIVE STUDY ON BEHALF OF THE FRANCOPHONE SOCIETY OF BONE MARROW TRANSPLANTATION AND AUTOGRAFTED LYMPHOMA. A RETROSPECTIVE STUDY ON BEHALF OF THE FRANCOPHONE SOCIETY OF BONE MARROW TRANSPLANTATION AND AUTOGRAFTED LYMPHOMA


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). Time between diagnosis and ASCT was 295 days (176-777). Patients received 1 (n=82, 44%), 2 (n=83, 44%), 3 or more (n=18, 10%), unknown (ND) (n=5, 2%) treatment lines before ASCT. Among the 138 B-cell lymphoma patients, 132 received rituximab before ASCT. Only 20 patients received prior radiotherapy. In all patients, ASCT was the first transplant. In 11 patients, ASCT was planned as part of a multiple graft protocol.

At the time of transplantation, 116 (62%) patients were in complete remission, 54 (29%) in partial remission, 13 (7%) in relapse or progression, and 5 (2%) ND. ASCT was documented in 186 (99%) patients. Median time to neutrophil and platelet (>50 Gigal/l without transfusion) recovery was respectively 11 days [10-12] and 19 days [14-32]. Infectious complications were found in 153 patients. One hundred (53%) patients had undocumented fever, 19 (10%) had sepsis, 150 (80%) had grade 1-4 mucositis during neutropaenia with a WHO toxicity grading of 2 (42%), 3 (39%) and 4 (19%). Colitis with a median duration of 7 days [5-10] was reported in 73 patients, with a maximum toxicity grading of 1-2 (n=43, 59%), 3 (n=21, 29%) or 4 (n=4, 6%) and ND in 5 patients. Only 2 (1%) patients had non-fatal hepatic sinusoidal obstruction syndrome. Pulmonary toxicity was reported in 33 (17.6%) patients with 8 cases of respiratory distress syndrome. Respiratory distress was fatal in one patient but occurred more than 6 months after ASCT and salvage treatment. Seven (3.7%) patients patients reported secondary cancers (all were solid tumors except one acute leukemia). Median follow-up was 17.1 months [11.3-29.5]. At the time of the study, 47 (25%) patients had relapsed. Cumulative incidence of relapse was 6.24% at 3 months and 17.31% at 12 months. At the end of the follow-up, 149 (79%) patients were alive. The main causes of death were relapse (n=15, 41%) and toxicity (n=16, 43%). Median overall survival (OS) was not reached and progression-free survival was 71.5 months [47-79]. 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 MICRORNAOME, PROTEOME AND METABOLOME OF EXOSOMES FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA
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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a clonal disease caused by the lack of glycosylinositol phosphatidylinositol 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 extracellular 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- and 3 without Eculizumab) using Total Exosome Isolation kit (Thermo Fisher). miRNA from exosomes were purified using Nucleo Spin miRNA Plasma Kit (Macherey-Nagel). miRNA expression was evaluated by plasma/serum focus miRNAs PCR panel V4 (Exiqon). Proteomic analysis of exosomes was performed at the OMICS core facilities. Untargeted metabolomic analysis was performed by using combination of gas chromatography and liquid chromatography (LC) with mass spectrometry (MS). Additionally, latest advances were used combining LC-MS-solid phase extraction-nuclear magnetic resonance (UPLC-QTOF_SPE_NMR) ‘on line’ for unequivocal structural elucidation of unknown metabolites.

Results: Mir-18-5p 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) and increased miR-15a-5p levels (0.50- and 3.12-fold respectively). Those proteins differentially expressed in patients and controls were related with the complement system and the immune response. We identified an increase in the plasma hemo-globin 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-4 region HG3 increased in 3.9-fold in the Eculizumab group vs without Eculizumab group, which could be related with the treatment. We identified quite few metabolites inside the exosomes, all of them associated with cell toxicity or immune response. The levels of Cholest-erol, HydroxyTerifiline-glucuronide and Dialcyl-glycerol decreased in 17.3, 17.6 and 19.4-fold, respectively in patients treated with Eculizumab. Interestingly, the Aminoethylphosphonicacid, Cholesterol and PGF2 increased 16.7-, 21- and 19.4-fold in patients with thrombosis.

Summary/Conclusions: Our study supports that exosomes contain material that may influence the pathological status of the PNH patients. In concordance, most of the proteins, miRNAs and metabolites are related with the complement system or the inflammatory response. In future experiments, some of the proteins, miRNAs and metabolites should be validated to define whether they could be considered biomarkers.

References

Severe chronic neutropenia as 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 extrahepatological 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 the 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 responsible for PID.

Results: From 2008 to 2016, 24 patients (13 males) with median age at last follow of 18yrs (range 28 mo-51y) had a complete work up for severe chronic neutropenia (Table 1). Ten/24 subjects (43%) were diagnosed as classical CN with ELANE mutation found in the majority (80%) of cases. Seven/24 (29%) were diagnosed as SN and the remaining 7/24 (29%) ON. A PID gene 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 ELANE in 2/7 patients. No mutation was found in the remaining 2. The phenotype of ON subjects is characterized by extra-hepatic autoimmune symptoms, by maturation block and by the frequent involvement of more than one hematopoietic lineage. This phenomenon may suggest to access to an enlarged genetic panel including PID genes for genetic diagnosis. An accurate immunological and genetic work may support diagnosis and management of these difficult patients.

Table 1.

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

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

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Background: Acquired aplastic anemia (AA) is a rare disease characterized by bone marrow failure. First-line treatment is either an allogeneic stem cell transplantation (alloSCT) or intensive immunosuppressive therapy (IST) consisting of Anti-Thymocyte globulin (ATG) and ciclosporin. Based on studies from the National Institute of Health, the Dutch guidelines for diagnosis and treatment of aplastic anemia recommend horse-derived ATG (ATGAM) as the preferred type of ATG. Patients who are refractory after first-line treatment with IST can be treated with second-line alloSCT or rabbit-derived ATG (Thymoglobulin) and ciclosporin. Response is defined as complete in case of normalization of blood values and as partial in case of transfusion independence and neutrophil count > 0.5 x 10^9/L. Overall survival was evaluated with the Kaplan-Meier method.

Results: In October 2016, 70 patients were registered in the NvHh registry. The start of treatment was 53 years (18-79) and median follow-up time was 18 months. Overall survival probability after 18 months was 87%. Fifty-nine patients were evaluable for a response at 6 months after treatment. Response was seen in 36 patients (61% (CI 49-73%)). Patients with a response at 6 months, had an overall survival probability of 94% at 12 months thereafter.

Summary/Conclusions: Response was seen in 36 patients (61% (CI 49-73%)). Patients with a response at 6 months, had an overall survival probability of 94% at 12 months thereafter. Six months after treatment with first-line ATGAM, 61% of the adult patients with acquired aplastic anemia is transfusion independent. Half of the remaining patients becomes transfusion independent after rescue treatment or after continuation of ciclosporin beyond 6 months.

Methods: In this study, we examined immune cell subset counts and immune globulins 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+CD28+ Tcell, CD4+ memory T cell and CD4+ naïve T cells. Simultaneously, we determined which factors influence immune reconstitution and analyzed the effects of different immune cell subsets on transplant outcomes.

Results: (i) The reconstitution of different immune cell subsets occurred at different rates after haplo-SCT. Monocytes were the first to recover, followed by CD8+ T and CD19+ B cells, and finally CD4+ T cells. Early CD4+ T cell recovery occurred at the expense of memory cells, whereas 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-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.
Background: Inherited bone marrow failure syndromes (IBMFSs) are a heterogeneous group of genetic disorders, with similar clinical presentations, resulting in complex diagnosis. Molecular characterization is essential in order to establish diagnosis, treatment and prognosis. Next-generation sequencing (NGS) techniques seem to be a useful platform for genetically defining different IBMFSs.

Aims: To design a NGS panel with the objective of making a specific, fast and cost-effective diagnosis for these pathologies.

Methods: We developed a NGS panel of 164 genes involved in different IBMFSs. A total of 120 samples have been processed. Patients were classified into two groups based on the clinical presentation: classified IBMFS (CBMFS) for those with a clinical picture typical of some of these disorders, and unclassified IBMFS (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 59.3%, coinciding with that described in the literature. Undiagnosed patients should be included in new research projects.

P581
APLASTIC ANEMIA PATIENTS WITH MONOCYTE-DOMINANT PNH CLONES HAVE A UNIQUE PRESENTATION AND ARE LESS RESPONSIVE TO IMMUNOSUPPRESSIVE THERAPY
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Background: Aplastic anemia (AA) is a bone marrow failure syndrome that can be successfully treated with either immunosuppressive therapy (IST) or allogeneic bone marrow transplantation (BMT). In ~50% of patients (pts) with AA, a clone deficient in glycosylphosphatidylinositol (GPI)-linked antigens—a paroxysmal nocturnal hemoglobinuria (PNH) clone—can be detected (Young, 2006). In recent years, highly sensitive techniques have been developed to test for PNH clones that have primarily focused on evaluating peripheral blood white cells. Neutrophils are routinely tested for expression of GPI with fluorescent eosin (FLAER); monocyes 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 >16 treated with IST at VGH, the tertiary referral centre for the Province of BC, between 11/09 and 10/15. All patients had central pathology review and metaphase cytogenetic analysis that confirmed a diagnosis of AA. High-sensitivity flow cytometry testing with a sensitivity of 0.1% was done on all patients

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 0-18y) diagnosed with DBA from all Dutch pediatric centers included in this study were 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 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 gene candidate, describing a novel gene defect 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-dosage-dependence, side effects, a weaning response, or a combination of these factors. Five patients (12.2%) were successfully transplanted with hematopoetic 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 hemoglobin values 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 underlying clinical heterogeneity. In addition, we have identified a novel gene defect 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.
to detect the presence of a PNH clone. Granulocytes, monocytes and erythrocytes were ingested with multi-colour flow panels including CD659 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 (Methyl prednisolone 1mg/kg/day x 10 days). CSA doses were adjusted to maintain whole blood trough CSA level of 200-300 ug/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 classic PNH phenotype. Six pts underwent BMT for primary non-response and 4 pts have died (2 post-BMT, 1 from complications of AA and 1 from breast Ca); 26 pts remain alive and well with a median follow-up of 48 mos (15-86). There were 17 pts (56%) with concordant granulocyte and monocyte clone sizes (Group 1), 4 pts (13%) had granulocyte-dominant disease (Group 2) and 9 pts (30%) had monocyte-dominant disease (Group 3). Group 3 pts were significantly more likely to have NSAA and showed a trend toward an inferior response rate to IST (Table 1).

**Table 1.**

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**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 29 YEARS**

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**Background:** Aplastic anemia (AA) is a rare, usually acquired disorder characterized by bone marrow failure with bl- or pancyclopenia and marrow hypoplasia. The classification into the three main subtypes is of prognostic and therapeutic relevance. Depending on disease severity, patient’s age, and the availability of a potential HLA-identical donor, different therapeutic strategies are favored. Immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine (CsA) is considered the initial standard treatment. A hematologic recovery is seen in up to 60-70% of the pts following horse-ATG (hATG) 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 review board of the University Hospital Essen, pts were included in the case series. Clinical data relative 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 4 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 seen in 68% 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=0.006). Irrespective 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 relapsed 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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Background: Stabilizing mutations of NOTCH1 have been identified in about 10% of chronic lymphocytic leukemia (CLL) cases at diagnosis, with a high frequency in unmuted IGHV (IGHV-UM)–immunocomprometery or advanced disease phase CLL, and have been associated with particularly unfavourable prognosis (Rossi et al, Blood, 2012; Del Poeta et al, Br J Haematol, 2013; Stilgenbauer et al, Blood, 2014); in CLL, all NOTCH1 mutations disrupt the C-terminal PEST domain and cause an accumulation of an active NOTCH1 isoform, resulting in a sustained pathway activation.

Aims: To identify molecular/biological features of NOTCH1 mutated CLL

Methods: The presence of NOTCH1 mutations was investigated by NGS. Gene expression profile (GEP) was performed by a one-color labeling strategy using the A44K platform. Specific gene/protein validations were performed by QRT-PCR, western blotting, flow cytometry and immunofluorescence. CLL-like MEC1 cell line was transfected with a vector containing a NOTCH1 isoform, resulting in a sustained pathway activation.

Results: i) A GEP comparing purified cells of 10 IGHV-UM cases (5 NOTCH1-mut; 15%±37% of NOTCH1 mutated alleles) selected nucleophosmin-1 (NPM1) and genes coding for several ribosomal RNPs as significantly up regulated in NOTCH1-mut cases. A higher expression of NPM1 and RNPs in NOTCH1-mut cases was validated in a wider independent series of 188 cases by QRT-PCR (76 NOTCH1-mut cases). In CLL, NPM1 expression was previously found higher in IGHV-UM cases (Rees-Unwin, Br J Haematol, 2009). In our series, no significant difference in NPM1 transcript expression was found by comparing IGHV-UM and IGHV-M cases, but NPM1 transcript expression was confirmed significantly higher in NOTCH1-mut than in NOTCH1-wt cases in the IGHM UM subgroup. ii) Western blotting in 11 CLL cases (5 NOTCH1-mut) confirmed a higher NPM1 protein expression in NOTCH1-mut cases, with a direct correlation with NOTCH1 expression (r=0.814). In NOTCH1-mut cases, the NPM1highsubpopulation, isolated by cell sorting, showed a higher NOTCH1 mutualional load than the NPM1low subpopulation. iii) EDTA treatment of 12 CLL cases (6 NOTCH1-mut) activated NOTCH1 signaling (Rand et al, Mol Cell Biol, 2000), as from HES1 and DTX1 induction, and up-regulated NPM1 and other RNPs. The same results were confirmed by co-culture of CLL cells with the JAGGED1-expressing M2-10B4 stromal cells. Inhibition of NOTCH1 signaling by gamma-secretase-inhibitor L-685,458 or by siRNA for NOTCH1 reduced NPM1 expression (Fig. A). iv) Previous studies identified MYC as a direct transcriptional target of NOTCH1 (Palomero et al, PNAS 2006) and, in turn, a transcriptional activator for both NPM1 and RNPs. ChIP assays on MEC1-cells, transfected with exogenous NICD, revealed increased NICD binding to the MYC promoter, along with higher expression of MYC, NPM1, and RNPs: Of note, after 48h culture, NOTCH1-mut CLL cases showed increased MYC transcript levels than NOTCH1-wt cases, MYC expression was further increased up to 4-5 fold in activation by EDTA or by stromal cells co-cultures (Fig. B). MYC silencing by siRNA efficiently reduced NPM1 transcript and protein expression. Moreover, CpG-ODN-2 treatment, to induce MYC overexpression, also increased NPM1 transcript and protein levels in CLL cells. w) NPM1 silencing by siRNA was able to reduce proliferation rates and cell size of both NICO-transfected cells and control cells. In keeping with a NOTCH1-driven regulation of cell growth/protein biosynthesis, activation of NOTCH1 signaling in 12 CLL cases (6 NOTCH1-mut) by EDTA or stromal cells co-culture, induced an increase in cell size.

Summary/Conclusions: NOTCH1 mutations in CLL are associated with the overexpression of MYC and MYC-related genes involved in protein biosynthesis including NPM1, which are allegedly responsible for cell growth and/or proliferation advantages of NOTCH1-mut CLL.

P584
CLL-LIKE B-CELL CLONES FROM MBLLO INDIVIDUALS PERSEVATE 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 lymphocytosis–MBLlo–) is a common finding in the general population. 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 clone like clonal B-cell population, using high-sensitive flow cytometry. A subset of them (n=49) has been followed for a median period of 84 months (range: 67-95 months). Besides physical examination and flow cytometry analyses, the most frequent CLL-related cytogenetic alterations [del(13q14.3)(D13S25), trisomy 12, del(11q)(ATM) and del(17p)(TP53)], and the NPM1 and/or RAS status were studied at baseline and during follow-up.

Results: A total of 64 CLL-like MBLlo clones (median size: 0.44 cells/ul, range: 0.027-66 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-in-fold overall increase in the number of B-cell clones after a 7 year follow-up vs baseline (median size: 1.22 cells/ul, range: 0.046-789 cells/ul; p<0.001); in line with this, most clones (45/64; 70%) increased their size, while the remaining 30% maintained stable or slightly decreased numbers compared to time 0. From the genetic point of view, only 8/32 (25%) clones were found to be altered and carry clonal cytogenetic alterations (del(13q)(D13S25) and/or del(17p)(TP53), between 7% and 10% of clones were being present in 7/8 cases and trisomy 12 in the remaining one. Strikingly, re-evaluation after 7 years showed 36/56 clones (64%); p<0.01 vs baseline) with cytogenetic alterations; again, the most common abnormality was del(13q)(D13S25) (34/36) followed by trisomy 12 (36) and del(7p)(TP53) (13/36). Myeloproliferative cellular association (p=0.05) was found between 2 it in the overall time in the size of these clones and the presence of cytogenetic lesions. Three subjects developed lymphocytosis (median: 5.3x109/lymphocytes/l; range: 4.1x109–5.9x109/l) after 7 years; in these cases the clone size increased sub-
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Background: The nuclear periphery, containing the IgH and Igk gene clusters, is a unique compartment comprised of inner nuclear membrane proteins and nuclear lamina. Previous genome-wide and cytological studies revealed the regulatory role for some of these nuclear proteins in higher level genome organisation and gene regulation. In particular, Lamina Associated Domains (LADs) were identified at the nuclear periphery as transcriptionally silent, gene-poor domains involved in the regulation of 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 IgM mutations in the pathogenesis of B-cell malignancies, we also tested whether nuclear lamina is involved in the pathogenesis of germinal centre lymphomas and chronic 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 vitro and in vivo.

Results: In a translational perspective, paired tissue microarray samples of diagnostic and transformed follicular lymphoma were analysed using immunohistochemistry and image analysis. Finally, comparative statistical analysis of CLL cohort patients was performed to test the impact of LMB1 expression on various clinical parameters in CLL.

Summary/Conclusions: In summary, here we show 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 events. We have shown that microenvironmental signals (e.g., IL-4) can increase BCR expression and signalling, and can partially reverse the effects of BCR-kinase inhibition. GAB1 and FOXP1 were previously shown to induce apoptosis of CLL samples resistant to ibrutinib. Cerdulatinib (cerd) is a selective IAP inhibitor that promotes BCR signalling by upregulating the expression of positive regulators FOXP1 and GAB1, and is being developed as a drug initiating BCR signalling in CLL cell lines 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 induced apoptosis in a synergistic manner. Here we investigated the effect of cerd in CLL samples expressing VLA-4 and assessed if cerd can promote resistance to therapies such as the BCL2-inhibitor venetoclax. We now extend these results to assess the effect of cerd on the regulation of BCR signalling in CLL cell lines in lymph nodes.

Methods: Eighteen primary CLL samples were treated with IL-4 +/-cerd (1μM) and expression of FOXP1, GAB1, PTEN, SOCS1 and SOCS3 assessed by immuno-blotting. 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 PTEN 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 PTEN and pSTAT6 (a positive control for IL-4 signalling). After 24hr IL-4 selectively increased expression of the negative regulators 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 IL-4 expression of other cytokines known to play a role in CLL biology (IL-6, IL-10, IL-15, IL-21 and IFNγ) 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 induced apoptosis in a synergistic manner. Here we investigated the effect of cerd on the regulation of BCR signalling in CLL cell lines in lymph nodes.

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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INSIDE-OUT VLA-4 INTEGRIN ACTIVATION IS MAINTAINED IN IBRUTINIB-TREATED CHRONIC LYMPHOCYTIC LEUKAEMIA EXPRESSING CD49D: CLINICAL RELEVANCE

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Background: VLA-4 (CD49d/CD29), a key molecule mediating cell microenvironmental interactions, can be activated via inside-out by BCR triggering in normal B cells. In chronic lymphocytic leukemia (CLL), nothing has been so far reported on activation mechanisms and this is even more important in ibrutinib, a drug that is known to determine an impairment of microenvironmental interactions with consequent shrinkage of tumor masses, and efflux of CLL cells into the blood stream.

Aims: To investigate in CLL the influence of VLA-4 expression/activation on ibrutinib response in-vivo.

Methods: VLA-4 activation was assessed by flow cytometry using conformation sensitive anti-CD29 mAbs (HUTS-21) and LDV-containing VLA-4 ligands, and measured as VLA-4 receptor occupancy (RO) (Chigaev et al. J Biol Chem, 2009). BCR engagement was performed using goat Fab(2) anti-human IgM. In-vivo studies were carried out on purified VLA-4+ CLL cells exposed in-vivo to ibrutinib. The clinical impact of VLA-4/CD49d expression on ibrutinib treatment was evaluated by measuring the kinetics of absolute lymphocyte count (ALC), the reduction of lymphadenopathy measured as sum of products of the diameters (SPD) % reduction from baseline, and the clinical outcome, as defined by progression free survival (PFS) in CLL patients treated with ibrutinib alone.

Summary/Conclusions: Altogether, these data suggest that during ibrutinib treatment CD49d+ CLL cells residing in tissue sites keep receiving BCR-mediated ITK-independent stimulus that, by inducing inside-out VLA-4 activation, result in enhanced cell retention, with consequent reduced lymphocytosis, relatively lower and/or slower nodal response, eventually leading to inferior outcome for CD49d+ CLL patients.
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 (ISRCTN12695854).

Aims: The IcICLLe trial was a single arm, multi-centre feasibility study of ibrutinib in two cohorts of CLL patients: (A) 20 treatment-naïve (TN) requiring treatment according to IWCLL criteria; and (B) 20 relapsed/refractory (RR). All patients received continuous oral therapy with ibrutinib (420mg once daily) from registration until disease progression. The primary endpoint of the trial was the proportion of patients achieving minimal residual disease (MRD) negative remission (depletion of CLL ≤0.01% in peripheral blood (PB) & bone marrow (BM)) within 6 months of trial treatment. Exploratory endpoints included the assessment of phosphorylation of intracellular kinases in the B-cell receptor pathway.

Methods: A panel of markers was assessed on PB & BM taken at screening, and 1 & 6 months. PB was also taken at baseline (0 hours), 4 & 24 hours, 7 & 14 days, and 2, 8 & 12 months. The phosphorylation of Syk pY348, Btk pY551, ERK1/2, Akt S473 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. Ibrutinib (10uM) 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 Syk, Btk, 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 1.5-2 fold increase in phosphorylation but the effect was abrogated within 1 month of ibrutinib therapy. Akt S473 phosphorylation was maintained after 6-12 months of therapy although the degree of phosphorylation decreased at later time points. Syk, Akt and ERK1/2 phosphorylation was unaffected by the addition of ibrutinib in vitro. The pattern of phosphorylation was found to be relatively consistent in responding patients. One patient with progressive CLL had sustained phosphorylation in all markers despite ibrutinib therapy.

Summary/Conclusions: The effect of ibrutinib on the phosphorylation of various kinases in the B-cell receptor pathway was analysed in real time. Syk continued to be phosphorylated over the course of treatment, which is logical as this kinase is upstream of BTK. That the degree of phosphorylation declined over time (even with stimulation) suggests a general inhibitory effect of ibrutinib on CLL cells. ERK1/2 phosphorylation is effectively blocked and there is partial reduction of phosphorylation of Akt S473. Combinations of Btk inhibitor with a Syk or PI3 kinase inhibitor may result in complete BCR blockade. Phosphorylation patterns may also act as an adjunct to ascertain the response to therapy.
While STAP-2 is expressed ubiquitously, STAP-1 has hematopoietic-specific of downstream molecules, including ERK, STAT5, BCL-xL and BCL2. The family via its SH2-like domain and enhances BCR-ABL activity leading to activation c-fms binding protein. Previously, we have demonstrated that STAP-2 binds to

**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) were developed to target the constitutively active oncoprotein BCR-ABL, which is expressed as a result of this translocation. TKI therapy has significantly improved patient survival, however predicting response to therapy is one of the unmet clinical challenges in CML. Moreover, TKIs are unable to target the leukemia stem cells (LSCs) which drive the disease; persistence of LSCs therefore remains a major obstacle to curing 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. This suggests that these may be important for the renewal, DNA damage repair, cell cycle and survival. These genes were validated in 60 samples from the SPIRIT 2 clinical trial [a multicentre phase III randomised trial comparing the TKI imatinib (400mg daily) versus Dasatinib (100mg Daily)] with 18 months follow-up data regarding molecular response to TKI treatment. Patients were stratified as good/intermediate/poor responders to TKI and the gene signature significantly differentially expressed was identified. These data highlighted the DNA repair genes as having potential predictive value, in particular, the 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+93+) compared to CD34+38-90+93- cells. Next, we investigated the ability of heliquinomycin (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 proved the potency of HQ and its synergistic action in combination with imatinib. We also investigated the changes in a panel 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. The findings of “omics” experiments are valuable for identifying novel pathways deregulated in CML. This combined with single cell ‘omics’ studies enables 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.

**Figure 1.**

**Summary/Conclusions:** In this study, we utilized CML mouse model and showed that STAP-1 is required for progression of CML. Our findings indicate that STAP-1 has an indispensable role in LSC maintenance, while normal hematopoietic stem/progenitors were not affected by STAP-1 deficiency. Although a majority of patients have a durable response to BCR-ABL tyrosine kinase inhibitors, the outcome of patients who fail the treatment due to primary or acquired resistance is still miserable. Our findings in mice and human suggest that STAP-1 could be a potential target for CML. Further analysis will be needed to clarify the molecular mechanisms by which STAP-1 regulates the progression of CML and maintains survival of LSCs.

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**TELOMERE SHORTENING IN CD34+38- BCR-ABL POSITIVE BONE MARROW STEM CELLS FROM NEWLY DIAGNOSED PATIENTS WITH CML CORRELATES WITH THE CLONE SIZE OF THE LEUKEMIC STEM CELL COMPARTMENT**

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**Background:** Chronic myeloid leukemia (CML) is a clonal stem cell disorder characterized by the BCR-ABL translocation. Previous work provides evidence that based on the size of the leukemic stem cell (LSC) clone within the CD34+38- population at diagnosis, chronic phase (CP) of CML can be stratified into early and late CP. Patients in late CP have a higher LSC burden going along with an inferior response to TKI therapy. Telomeres shorten with each

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**SIGNAL TRANSDUCING ADAPTOR PROTEIN-1 (STAP-1) MAINTAINS CHRONIC MYELOID LEUKEMIC STEM CELLS**

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**Background:** Signal transducing adaptor protein (STAP) -2 was cloned as a c-fms binding protein. Previously, we have demonstrated that STAP-2 binds to BCR-ABL, which is constitutively activated in chronic myeloid leukemia (CML), via its SH2-like domain and enhances BCR-ABL activity leading to activation of downstream molecules, including ERK, STAT5, BCL-xL and BCL2. The family of STAPs includes STAP-1, identified as a c-kit interacting protein, and STAP-2. While STAP-2 is expressed ubiquitously, STAP-1 has hematopoietic-specific expression in mice. It is still unknown whether STAP-1 plays a role in CML,
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 recently completed study to determine if BCR-ABL+ LSC vs. 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 NCT00825266 study (Nordic CML Study Group) were retrospectively analyzed. Thirteen patients (median age at diagnosis 59.5 years, range: 32.0-72.0 years) and 2 patients (14% who were optimal responders to TKI and remained in MMR for at least 21 years) were available for 14 patients. Of those, 2 (14%) belonged to the Sokal high risk group, 5 (36%) to the intermediate and 7 (50%) to the low risk group. CD34+38- cells sorted from bone marrow samples were tested with the standard FISH method using dual fusion dual color/ dual fusion painting following standard procedures. After capturing the BCR-ABL staining using confocal microscopy, samples 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 17.9 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 59.5% ± 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 of telomeric data with Sokal (R²=0.69, 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.

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**GENOMIC CHARACTERIZATION OF CML AT DIAGNOSIS REVEALS PREDICTIVE 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 recent reports to date which use next-generation sequencing (NGS) to look for somatic mutations - other than those affecting kinase domain of BCR-ABL - at the time of diagnosis (Dx) which could have a prognostic/predictive value.

**Aims:** We aimed to analyze the spectrum of somatic mutations in two groups of CML patients with clinically different disease course: first group (BP) comprised of 11 patients who progressed to BP-CML despite treatment with TKI and/ or allo-HSCT (one patient) and died (paired samples from Dx and BP were analyzed); second group (MMR) included Dx samples from 36 patients who achieved major molecular response (MMR) 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 employed. More than 1200 genes implicated in human cancer were included. Common variants (>1%) gathered in large genomic databases and our internal laboratories.

**Results:** We analyzed the spectrum of somatic mutations in two groups of CML patients: first group (BP) comprised of paired samples from 11 CML patients who progressed to BP and died despite treatment with TKI. Median age at diagnosis was 53y (range 26-77), median time to progression for 9 patients (2 were diagnosed in accelerated phase or BP) was 17.5 months (mo) (range 4-108) and median survival was 22 mo (range 10-116). None of those patients harbored BCR-ABL1 mutation at the time of Dx and progression to BP-CML, 4 patients had additional chromosomal alterations at progression to BP including two frequent (trisomy 8 and monosomy 7). Targeted enrichment followed by NGS allowed us to achieve deep coverage (>80% ge50). Median number of rare variants was 26 (range 18-38) and 29 (range 23-32) for Dx and progression to BP samples, respectively. In the BP group, 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 the BP group (54%, 6/11) mutations in these genes (excluding IDH2, detected only in 1 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: 73mo; range 48-128). In MMR group, the median number of rare variants was lower than in BP patients (2/36, 5%) frameshift mutation in ASXL1 (p.Gly643_Gly644fs) was detected, identical as in one of BP patients. Additionally, one patient harbored RUNX1 mutation (p.Arg201Cin) which was not detected in the BP group.

**Summary/Conclusions:** Our results provide new insights into the already complex genomic landscape of BP-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.

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**INCREASED INDOLEAMINE 2,3-DIOXYGENASE (IDO) ACTIVITY IN EARLY CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA (CML-CP) IS REDUCED BY NILOTINIB THERAPY AND PREDICTS MOLECULAR RESPONSE**

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**Background:** Indoleamine 2,3 dioxygenase (IDO1) is the rate-limiting enzyme in the metabolism of the essential amino acid tryptophan (TRP). IDO1 is induced mainly by interferons during infection and inflammation. Strong IDO1 activity depletes tryptophan, which results in reduced T cell activation and proliferation as well as expansion of immunosuppressive regulatory T cells. Dere-activation of IDO1 activity has been linked to cancer immune evasion, but its role in chronic phase (CP) CML has not been investigated in detail.

**Aims:** Determination of IDO1 levels and activity in plasma CML-CP patients in the course of tyrosine kinase inhibitor therapy and their correlation with clinical and immunological parameters as well as molecular response.

**Methods:** A large panel of cytokines and components of the IDO-pathway (soluble ID1= sIDO1 and kynurenine/tryptophan ratio=kyn/TRP as a product of IDO1 activity) as well as various leukocyte populations such as plasmacytoid dendritic cells (pDC) were analyzed alongside the prospective pan-European ENEST 1st clinical study (NCT0161177). This study included 64 patients on chronic phase (CP)-CML patients that were subsequently treated with 300mg BID nilotinib and longitudinally analyzed at months 6 and 12 of therapy. Molecular responses were quantified in central EUTOS reference laboratories.

**Results:** The soluble IDO (sIDO1) levels and KYN/TRP ratio are significantly upregulated in newly diagnosed CP-CML and drop during nilotinib therapy. sIDO1 levels significantly correlate with increased KYN/TRP, suggesting increased IDO1 activity at diagnosis. Increased sIDO1 is linked to a pro-inflammatory status in CML patients, as it positively correlates with increased serum neopterin levels as well as to various other pro-inflammatory markers, such as IFN-γ, IL-10, IL-17A, TGF-β and sVCAM-1. IDO1 is frequently mutated in selected genes, IDH1 activity (KYN/TRP) negatively correlates with the proportion of pDC, the main producers of IFN-α. Interestingly, a higher KYN/TRP is linked to superior molecular response, as demonstrated by a significant correlation
of the KYN/TRP ratio to BCR-ABL transcript levels. Patients having a high KYN/TRP ratio (> mean +2SD of post therapy levels) reach deep molecular response rates (i.e. MR4.5) significantly earlier and at higher rates. Moreover, combining KYN/TRP with sCD62L levels, a recently identified predictive biomarker, resulted in a score robustly predicting the odds of achieving deep molecular response.

Summary 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 molecular response and survival. As a result, to implement testing for KYN/TRP would be highly desirable, therefore, to predict and monitor drug concentrations and monitoring the kinetics of mutant subclones covering drug-resistant mutations in the routine diagnostic surveillance to provide a basis for optimized clinical management of patients treated with ponatinib.

Methods: Aims: 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 constellation expected to display high resistance to ponatinib. To assess the anticipated responses to ponatinib in vitro, we have introduced all BCR-ABL1 CMs into Ba/F3 cells using a recently published transposon-based approach (Byrgazov et al., Oncotarget 2016, 7(47);78083-78094), and IC50 values for each CM were determined.

Results: Most CMs involving sites with no previous evidence in implication in resistance to ponatinib displayed IC50 values below 10 nM. This eF_{cave} 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 eF_{cave} 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 helpful, therefore, to implement testing of the eF_{cave} in clinical practice 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.

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IS THERE EFFECTIVE IMMUNE SURVEILLANCE AGAINST CHRONIC MYELOID LEUKAEMIA? NO

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Background: Immune surveillance refers to a process whereby the innate and adoptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (GvHD) and whether there is a 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 adoptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (GvHD) and whether there is a 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.

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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 TKI resistance is the acquisition of mutations in the BCR-ABL1 tyrosine kinase domain (TKD) affecting TKI binding. The third-generation TKI ponatinib exerts strong anti-neoplastic effects even in advanced CML stages and is capable of suppressing the kinase activity of BCR-ABL1 carrying any single mutation including T315I. Nevertheless, resistance to ponatinib can evolve in sub-clones carrying BCR-ABL1 variants with two or more mutations on the same allele, if the IC50 values for this TKI exceed the maximum achievable effective plasma levels (eF_{cave}). These so-called compound mutations (CMs) are associated with increased oncogenic potential in comparison to individual mutations, and represent a powerful mechanism of potential resistance to all currently available TKIs. The occurrence of compound mutations has been linked particularly to sequential treatment with different TKIs, and the identification of their responsiveness to ponatinib is of paramount importance for the subsequent clinical management.

Aims: 1. To determine the spectrum of highly TKI-resistant CMs. 2. Measure the responses of BCR-ABL1 CMs to ponatinib

Methods: We have established a BCR-ABL1 protein model facilitating assessment of the presumptive impact of 27 different CMs involving important functional sites of the BCR-ABL1 TKD, and including constellation expected to display high resistance to ponatinib. To assess the anticipated responses to ponatinib in vitro, we have introduced all BCR-ABL1 CMs into Ba/F3 cells using a recently published transposon-based approach (Byrgazov et al., Oncotarget 2016, 7(47);78083-78094), and IC50 values for each CM were determined.

Results: Most CMs involving sites with no previous evidence in implication in resistance to ponatinib displayed IC50 values below 10 nM. This eF_{cave} 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 eF_{cave} 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 helpful, therefore, to implement testing of the eF_{cave} in clinical practice 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.

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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 comply with local ethical and privacy regulations. The CTS dataset consisted of 441,332 recipients of kidney (N=355,606), liver (N=47,846) and heart (N=37,880) transplants. Amongst kidney transplant recipients the standardized incidence ratio (SIR) for developing CML was 1.54 (95% confidence interval, 1.1, 2.1; p<0.01) representing 39 cases in 1,682,491 person-years at risk (53 cases, 36 observed). 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 the 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 computed tomography (CT)-angiography. Ionizing radiations are a proved cause of CML which might explain the increased SIR.

Results: Our data, 25 excess cases of CML in 2,038,339 person-years at-risk in the Italian CTS dataset, suggest the magnitude of immune-surveillance do not support the hypothesis immune surveillance operates to an important extent to prevent CML in humans.

Summary/Conclusions: Consequently, the anti-leukaemia effect associated with allografts 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 LEUKAEMIA 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 has 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, the classification and reporting of detection level variants is not uniform.

Objective: To compare the results of mutational analysis of the BCR-ABL1 KD with the deep sequencing technology MinION, in patients affected by CP-CML and MR4+MR4.5/5.

Methods: We assessed the BCR-ABL1 transcript in 86 patients afferent to the Chronic Myeloid Leukemia Study Group (CMLSG) study.

Results: 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 copies of ABL1, while by the automated method 81 samples (94.2%) reached >100,000 copies of ABL1. We assessed the BCR-ABL1 transcript in 86 patients afferent to the Chronic Myeloid Leukemia Study Group (CMLSG) study.

Results: Two sequencing runs were performed with the two different pools of patients: the first lasted eight hours and was carried out on the Group 1, while the second run included the Group 2 and lasted 24 hours to achieve a deeper sequencing. Sequencing results showed that 100% of ABL1 from exon 2 to 10 was covered and that the mean of the sequencing depth was around 150x and 100x for the two methods respectively, any kind of sequence was never found below 50X. We found 10 BCR-ABL1 KD mutations in 9 patients belonging to the Group 1 (one case showed compound mutations). Notably, almost all mutations had a high allelic ratio. Despite a high degree of sequencing, MinION data analysis on the Group 2 was able to detect mutation only in a ALL case. Results from MinION and SS showed 92% concordance in all cases included in this study. Notably, mutations that were initially undetectable by SS became evident thanks to the indications coming from MinION analysis.

Summary/Conclusions: Our findings demonstrate multiple advantages by using MinION approach, first of all the sensitivity: our comparison of MinION to SS identified mutations below the detection limit of SS (generally estimated around <20%) in 222 among the mutated cases, including mutations known to be clinically important. Another point on the side of the nanopore technology is the costs profile. Therefore, the main advantage of this technology is to allow a more efficient and sensitive analysis than SS at very competitive costs. In conclusion, we demonstrated that MinION is suitable for employment in hematology laboratory for detecting BCR-ABL1 KD mutations in Ph+ leukemias.

P599 THE AUTOMATED MOLECULAR TECHNIQUE “ULTRA” ALLOWS A SENSITIVE AND ACCURATE BCR-ABL1 QUANTIFICATION IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKAEMIA

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Background: The chronic myeloid leukaemia (CML) is characterized by the presence of the Philadelphia chromosome and the BCR-ABL1 fusion gene. The induction 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% ≤10% IS), the MR3 (<0.1%) and the deep molecular response (MR4<0.01%, MR5<0.006%, 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. Amongst the NGS techniques (Ion Torrent, 454 GS FLX, SOLiD Sequencing, Illumina MiSeq, 10x Chromium, Oxford Nanopore Technologies Ltd., Oxford, UK) the nanopore technology is the “classical” 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 subgroup.

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 treatment levels (from <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 MR4+MR4.5 (32000, 64 000, 128 000) and the MR5 (320 000, 640 000, 1 280 000). 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 (K Cohen=0.973 p<0.001) and statistical differences in deep molecular responses 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 large series of patients the automated and manual molecular methods, applied in 4 different laboratories, resulted comparable in classification of patients in “molecular classes”. The advantage of the Ultra technique is represented by the higher number of detected ABL1 copies and the easier standardization.

P600
ROLE OF THE AURORA KINASE A/PLK1 AXIIS INHIBITION IN RESTORATION OF CELL GROWTH CONTROL OF CHRONIC MYELOID LEUKEMIA PROGENITORS

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Background: ENESTfreedom (NCT01784068) is evaluating the ability to stop NIL and regain MMR in TFR in pts with a sustained deep molecular response (MR) on frontline NIL. Previous results from ENESTfreedom showed that 51.6% of pts (98/190) who attempted TFR remained off treatment and in major MR (MMR; BCR-ABL1 ≤ 0.1% on the International Scale [IS]) at 48 wk.

Aims: To analyze updated TFR data and predictive factors for remaining in TFR in ENESTfreedom.

Methods: Eligible pts had CML-CP with b2a2 and/or b3a2 BCR-ABL1 transcripts, ≥2 y of frontline NIL, and MMR (BCR-ABL1 ≤ 0.0032%) prior to enrollment. All pts provided informed consent. After enrollment, pts continued NIL for ≥2 y, remained in the study until relapse and/or discontinuing from study medication. All pts provided informed consent. After enrollment, pts continued NIL for ≥2 y, remained in the study until relapse and/or discontinuing from study medication. All pts provided informed consent. After enrollment, pts continued NIL for ≥2 y, remained in the study until relapse and/or discontinuing from study medication.

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. Three pts who were in TFR at 48 wk lost MMR by 96 wk, and 2 additional pts discontinued from the study between 48 and 96 wk without losing MMR. Among pts with low, intermediate, or high Sokal risk at diagnosis, 39/62 (62.9% [95% CI, 49.7% - 74.8%]), 25/50 (50.0% [95% CI, 35.5% - 64.5%]), and 9/28 (32.1% [95% CI, 15.9% - 52.4%]), respectively, remained in TFR at wk 49 (Sokal risk scores were missing for 50 pts). Among pts with continuous MR4 in all phases, of 88 pts who remained in TFR at 48 wk, 81 (91.8%) remained in TFR at 96 wk (91.8% [95% CI, 81.3% - 95.2%]) and 87 (98.9%) regained MMR and the remaining pt left the study 7.1 wk after NIL reinitiation without regaining MMR; 81 of 88 pts (92.0%) regained MR4-5 by the data cutoff. Among pts remaining in TFR for ≥48 wk (n=100), adverse events (AEs) were less frequent during the second vs the first 48 wk of TFR; 2 (2.0%) and 1 (1.0%), respectively. No pts had cardiovascular AEs during the 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 predictors for remaining in TFR were identified. Pts with low Sokal risk and pts with continuous MR4-5 in the consolidation phase tended to have higher TFR rates than other pts, although these results must be interpreted with caution due to the small number of pts in some subsets and the wide 95% CIs. Additionally, the biological explanation for an association between Sokal risk score at diagnosis and a subsequent ability to remain in TFR is unknown. These results support TFR as a valuable option for pts in sustained DMR on frontline NIL.

P602
RESPONSE DIFFERENCES IN THE BCR-ABL1 E13A2 AND E14A2 VARIANTS MAY BE A TECHNICAL QPCR ARTIFACT

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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 Myers against Cancer (EAC) qPCR assay has been 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, amplions can 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 discrepancies in clinical decision-making, where e13a2 predicts a faster and deeper treatment response. However, no difference in overall survival has been observed and the issue remains controversial.

Results: Transcript levels from 124 BCR-ABL1 positive patient samples were determined using the EAC qPCR assay (median: 0.08% IS, range: 0.001–159% 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 discrepancies in clinical decision-making, where e13a2 predicts a faster and deeper treatment response. However, no difference in overall survival has been observed and the issue remains controversial.

Figure 1.

Summary/Conclusions: When we compared the BCR-ABL1 levels using qPCR and ddPCR, we observed a discrepancy between the e13a2 and e14a2 breakpoints. 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 the e13a2 variant. Thus in qPCR analyses using the EAC protocol, it may be at least on suboptimal 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.

Table 1.
29%/23%. Among CP-CML pts with no prior AEs who had a prospective dose reduction, 17% (11/63) had a first AOE occurring after Oct ’13.

Summary/Conclusions: Long-term 5-y results from PACE demonstrate that irrespective of dose reductions, ponatinib continues to show deep, lasting, clinically meaningful responses over time in heavily pretreated pts with CP-CML. Achieving early cytogenetic response and deep reduction in BCR-ABL1 levels was associated with improved survival 4 yrs past landmark, demonstrating the prognostic value of early and deep response to ponatinib.

P604

LONG-TERM FOLLOW-UP IN VERY ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB FRONTLINE


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Background: Very elderly (>75 yrs) people are a substantial proportion of chronic myeloid leukemia (CML) patients that sometimes receive Imatinib (IM) at reduced doses based on physicians’ judgment. However, data on long-term follow-up of these patients are still lacking.

Aims: To investigate the treatment response and outcome in a cohort of very elderly patients with newly diagnosed CML in chronic phase.

Methods: We revisited in a retrospective database 263 CML, elderly patients aged >75 years and diagnosed from 2/2002 to 1/2016 and treated with IM frontline; among these, 121 patients (46%) were older than 80 yrs.

Results: Median age at diagnosis was 78.5 yrs [interquartile range (IQR) 76.3 – 81.3]. Sokal Risk at diagnosis was low in 1 patient (0.4%), intermediate in 171 (68%), high in 40 (15.3%), while standard risk was not available in 13 patients. As regards comorbidities, 63 patients had no or 1 concomitant disease, 147 patients 2 or 3 and 53 patients (20.1%) 4 or more. Median interval from diagnosis to IM start was 0.8 month (IQR 0.3-1.6); the initial IM dose was 400mg/day in 180 (68.4%), 530mg/day in 67 (25.5%) and <300mg/day in 16 (6.1%) patients. According to WHO dose reduction was clinical and/or hematological abnormalities and 400mg/day was the 1st dose in 70% of patients. Median duration of IM treatment was 3.8 years and diagnosed from 2/2002 to 1/2016 and treated with IM frontline; among these, 121 patients (46%) were older than 80 yrs.

Summary/Conclusions: The long term follow-up of very elderly CML patients treated with IM 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.

P605

IMPACT OF ARTERIAL THROMBOTIC EVENTS ON THE LONG-TERM OUTCOME OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH Nilotinib: A MULTICENTER STUDY WITH NILOTINIB


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Background: Nilotinib has shown better efficacy compared to imatinib, but it has been associated to a higher incidence of arterial thrombotic events (ATEs). One of the most important concerns is the long-term outcome of patients treated with nilotinib.

Aims: To investigate the characteristics of ATEs and their impact on the long-term outcome of CML patients treated with nilotinib first-line.

Methods: We analyzed 345 patients ≥ 18 years of age with CP CML enrolled in clinical trials of the GIMEMA CML WP investigating nilotinib as first-line treatment. Patients were treated with: nilotinib 400mg BID (n=173); rotation of nilotinib 400mg BID / imatinib 400mg OD (3-month periods for each drug)(n=123); nilotinib 300mg BID (n=149). The median follow-up was 58 (22-82) months. The median age at CML diagnosis was 53 (18–86) years. We analyzed the rate, type, management, and outcome of ATEs; moreover, we compared the molecular response rates and the long-term outcome of CML patients treated with nilotinib.

Results: Overall, 84 (7.6%) patients had ATEs during treatment with nilotinib. The median age at CML diagnosis of these patients was 64 (43-85) years, and the median age at ATEs was 67 (47-89) years. The median duration of nilotinib treatment at ATE was 25 (1-78) months. ATEs were: 14 coronary dis...
ease (including 7 acute myocardial infarction), 8 PAOD, 4 carotid stenosis (asymptomatic), 2 avascular necrosis of the femoral head, 1 optic artery ischemia, 1 arteritis 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 71% 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 (MR3: 260/315, 83%; MR4: 113/315, 64%). The 5-year progression-free survival and overall survival were similar in patients with or without ATEs (PFS: 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.

Assessment of chronic renal injury in patients with chronic myeloid leukemia (CML) 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 TKIs 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 receiving 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 mediastinal mass (HR=3.6, 95% CI 1.5-13, 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.
discontinued TKIs, a preliminary analysis showed that 80% of patient with BCR-ABL1>0.468 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:**

- Total of 26 patients (44.8%) proceeded to allogeneic bone marrow transplant, 26% (n=6) of which only received TKI prior to transplant compared to 76.9% (n=17) of patients treated with intensive induction chemotherapy, outcome remains dismal.
- Of interest 9/18 patients (50%) achieved MR4 by 12 months, and 66.7% (n=12) obtained MMR at 6 and 12 months, and they were done both previous to the dose, and 2 hours after.

**Figure 1.** Overall survival in the era of TKI in management of BP-CML.

**Table 1.**

<table>
<thead>
<tr>
<th>Lymphocytes Baseline</th>
<th>CD3 Baseline</th>
<th>CD4 Baseline</th>
<th>CD8 Baseline</th>
<th>NK Cell Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (x 10^9/L)</td>
<td>1.78 (0.92-2.24)</td>
<td>0.40 (0.12-1.48)</td>
<td>0.80 (0.1-1.29)</td>
<td>0.20 (0.02-0.77)</td>
</tr>
<tr>
<td>Percentage</td>
<td>27.4 (14.5-23.1)</td>
<td>11.6 (6-26.8)</td>
<td>11.6 (6-26.8)</td>
<td>11.6 (6-26.8)</td>
</tr>
</tbody>
</table>

**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 CCyR 1.4 years (0.2-12). Of interest 9/18 patients (50%) achieved MR4 by 12 months. There were 3 study discontinuations because of toxicity (16%). Table 1 shows the median number of the most relevant lymphocyte populations in the pre-dose sample at baseline. Table 2 shows that the absolute number of CD8 cells was significantly superior at baseline in those patients having a MMR at 3 months, with a trend in the same direction of absolute lymphocyte count and percentage. There were no significant associations with response when considering CD4 T cells, NK cells, or the degree of mobilization after dasatinib dose either in total lymphocyte 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 2.**
Summary/Conclusions: Our study shows that in patients treated with imatinib and with late warning responses, switching to dasatinib induced MMR in 2 out of 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 obtention 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) represent 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, ANKR55/D5/MPK3K1, PDGFC, LYP/PLAL1, RSPO3, and FAM13A1 genes. The uGRS was based on the sum of the risk alleles within the set of selected SNPs.

Results: uGRS, 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 pre-diabetes. Pre-diabetes/diabetes-free survival was significantly higher in patients with an uGRS below 10 compared to higher scores (100% vs 18%, p=0.004) (Figure). Each increment of 1 unit on the uGRS caused a 42% increase in the pre-diabetes/diabetes risk (HR=1.42; CI: 1.04-1.94; p=0.026).

Summary/Conclusions: Impaired fasting glucose (IFG) and type 2 diabetes (T2D) represent adverse events in Chronic Myeloid Leukemia (CML) patients treated with nilotinib. A genetic risk score (uGRS) for the prediction of insulin resistance, consisting of 10 multiple single-nucleotide polymorphisms (SNPs), has been proposed. We evaluated the uGRS predictivity in 45 CML patients treated with nilotinib.

P611
THE EUROPE AGAINST CANCER PROTOCOL FOR BCR-ABL P210 TRANSCRIPT MEASUREMENT MAY OVERESTIMATE RESULTS FOR E13A2 AND E14A2
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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 Ade-laide 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 recalculcation, showed very good concordance with Adelaide (100% results of e14a2 and 88% of e13a2 within 2-fold of reference laboratory).

Summary/Conclusions: In the EAC protocol, the e14a2 transcript amplifies less efficiently than e13a2. Since commonly used plasmids, including ERM-AD623, are based on e14a2, the standard curve is being shifted towards the latter cycles. It leads to overestimation of e13a2 by mean factor of 4.5 (over 0.5 log), which could be clinically significant. The reports of worse outcome of e13a2 patients are probably caused by this artifact, which can be easily eliminated by implementing an additional forward primer to EAC protocol. This overestimation cannot be detected in case of lab to lab validation when two centers are using EAC protocol. In case of method validation in Adelaide, those differences were not as obvious as well. The shift of 4.5 (fig. 1) means that results are 2.25 times different from the perfect concordance line and could easily fit into accepted 2-fold and 3-fold compartments. The CF calculated by Adelaide would depend on the percentage of each transcripts among the exchanged samples. The observed artifact should be also taken into consideration in clinical trials that rely on surrogate endpoints such as molecular response level at certain time points. Uneven transcript variant distribution between compared groups may lead to improper conclusions.
Enzymes and sickle cell disease

P612

ESTABLISHMENT OF IN VIVO AND IN VITRO MODEL OF X-LINKED SIDEROBlastic ANEMIA

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Background: Congenital sideroblastic anemia (CSA) is a inherited hematologic anemia characterized by the presence of bone marrow ring sideroblasts, reflecting excess mitochondrial iron deposition. The most common form of CSA is X-linked sideroblastic anemia (XLSA), which is attributed to mutations in the X-linked gene erythroid-specific 5-aminolevulinate synthase (ALAS2). ALAS2 resides on chromosome X and encodes the enzyme that catalyzes the first and rate-limiting steps in the heme biosynthesis pathway in erythroid cells. This pathway converts glycine and acetyl-coenzyme A to 5-aminolevulinic acid (ALA), which requires pyridoxal 5'-phosphate (PLP) as a cofactor. Although PLP has been used for treating XLSA, a marked proportion of patients with XLSA remain refractory to treatment (Ohba et al. Ann Hematol 2013). Thus, there is a need to establish a model of XLSA to reveal the detailed molecular mechanism contributing to RS formation as well as to explore novel therapeutic strategies.

Aims: We explored to establish a novel model of XLSA by CRISPR/Cas9-based genome editing.

Methods: We targeted the GATA-1-binding region of intron 1 of the human ALAS2 gene based on both in vivo mice and human induced pluripotent stem cell-derived endoderm (HiDEP) cells (Kurita et al. PLoS One 2013). The mutation diminished the binding of transcription factor GATA-1, which would lead to decreased transcription of the ALAS2 gene, thereby causing XLSA (Kaneko et al. Haematologica 2014). Western blotting and quantitative chromatin immunoprecipitation (ChIP) analysis were performed using antibodies against GATA-1 (D52H6, Cell Signaling Technologies) and TAL1 (C-21, Santa Cruz). For transcription profiling, Human Oligo chip 25K (Toray) was used. Gene ontology (GO) analysis was performed with Genecoids (http://genecoids2.dacya.ucm.es/).

Results: We first generated a founder female mouse lacking the intron 1 enhancer region of Alas2, including the GATA binding domain (Alas2Δint1/X). Whereas the heterozygous Alas2Δint1+/X mice were viable and did not show anemic phenotype, hemizygous deletion (Alas2Δint1/X) in male mice led to an embryonic lethality, suggesting that this sequence is indispensable in the context of mice. As an alternate approach, we established a clonal line with HiDEP cells, which harbored 19-bp deletion within the intron 1 enhancer region of Alas2, including GATA binding domain. Whereas wild-type HiDEP cells exhibited 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 GATA1 protein in the XLSA clone, 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- and down-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 HBB) as well as genes involved in iron/heme metabolism (ALAS2, transferrin receptor, TFR, copper/iron oxidase: CPOX, and mitoferrin 1: MFRT1). GO analysis revealed significant enrichment of cellular iron homeostasis (p=0.018), regulation of transcription (p=0.0021), and innate immune response (p=0.0018), implying that heme was involved in various biological processes in erythroid cells. Interestingly, ALA treatment significantly improved compensated heme production as well as downregulation of globin genes observed in the XLSA clone, suggesting that ALA may represent a novel therapeutic option for PLP-refractory XLSA.

Summary/Conclusions: The XLSA model established from HiDEP cells can be used as an important tool for clarifying the molecular etiology of XLSA and to explore novel therapeutic strategies.

P613

BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY FOR COLD AGGLUTININ DISEASE: 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 cold agglutinin-fixated circulatory symptoms. Pharmacological therapy, although not indicated in patients with very mild disease, seems required in a majority of cases. Corticosteroids are ineffective. Rituximab monotherapy has resulted in approximately 50% response rate and 1-year median response duration. Fluadrabine and rituximab combination therapy showed 70% response rate (20% complete responses) and very long response duration, but considerable toxicity.

Aims: We wanted to investigate whether bendamustine and rituximab combination therapy can result in favorable response rates and duration with an acceptable toxicity profile.

Methods: We conducted a prospective, uncontrolled multicenter trial with 16 participating hospitals from Norway, Finland and Denmark. Essential inclusion criteria were verified CAD with symptomatic anemia and/or severe cold-induced circulatory symptoms. Eligible patients received 4 cycles of rituximab 375mg/m2 day 1 and bendamustine 90mg/m2/day 1-2 with 28 days interval. Outcomes were evaluated into complete response (CR), partial response (PR), complete response with 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 8.9 g/dL (range, 4.5-14.8), bilirubin 45μmol/L (lactate dehydrogenase (LDH) 468 μL, haptoglobin undetectable, IgM 4.1g/L (1.0-2.72), CA 125 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 a median of 4 of the responders; 4 g/dL in patients achieving CR and 3.9 g/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 from response, consistent with a much longer expected response duration. Neutropenia grade ≥3 occurred in 14 patients (32%), of which 8 (18%) had grade 4. Three patients (7%) experienced 1-3 episodes of febrile neutropenia, which was readily manageable. Non-hematologic toxicity occurred in 17 patients (39%), mostly consisting of mild nausea or rash. Three non-neutropenic serious adverse events (SAE) were recorded; 1 was considered probably 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.
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 isomorph of PK (PK-R) and is in clinical development for the treatment of PK deficiency.

Aim: To evaluate the effect of AG-348 treatment on PK-R enzymatic function, RBC metabolism and deformability.

Methods: Observational case-control study, approved by the Institutional Review Board. All patients gave informed consent. Enrolled patients (N=15) were adults, transfusion-independent and compound heterozygous or homozygous for mutations in PKLR.

Results: Baseline protein level analyses suggests that a certain level of PK-R protein is required for cells to respond to AG-348 treatment whereas AG-348 effectively upregulates PK-R enzymatic activity and translation with AG-348 prior to incubation resulted in residual activity 1.4 to >10-fold higher than residual activity of vehicle-treated samples. Baseline enzyme levels 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 in PK-deficient RBCs, as ATP levels in PK-deficient cells increased 2.3, range, 1.2-7.1). ATP levels in PK-deficient cells increased enzymatic activity in all patient cells after 24 hours (mean increase 1.4-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 increases upon AG-348 treatment (mean increase 1.4-fold, range 1.2-2.2) similarly to control cells (mean increase 1.6 fold, range 1.4-1.8). Generally, PK-R residual activity and response to ellyptocytosis, was identified in 6 patients from 5 different unrelated families. There were also 42 patients to ellyptocytosis, was identified in 6 patients from 5 different unrelated families. The following therapeutics were used in 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 worth noting that almost 10 of the 13 undiagnosed patients had been oriented as unclear membraneopathy.

Conclusions: According to the results, there is a high genetic heterogeneity in patients with RBC membrane disorders, as almost each family carries a unique mutation that is not observed in any other no related family. The present study reveals the usefulness of NGS panel, which allows the molecular diagnosis of almost the 90% of the patients and it would avoid misdiagnoses. The diagnosis could lead to splenectomy in cases of mild disease, but it didn't suggest any specific RBC membrane pathology.

Results: A total of 74 pathogenic variants leading to RBC membrane disorders were identified, of which 14 had already been reported as disease causing. Of the remaining 60 variants, 42 had never been identified neither by 1000G or ExAC projects and therefore are novel mutations. Beta-spectrin, ankyrin and alpha-spectrin were the proteins that gathered most part of the mutations, we identified 23 variants in SPTB, 20 variants in ANK1 and 16 variants in SPTA1 (36/74) of the identified variants were missense changes, mostly from SPTB gene (11 genes), while a 38% (28/74) of the variants were nonsense mutations, mostly from SPTA1 (12 variants) and SPTB (9 variants). Of special interest, only 2 variants were identified in more than one unrelated family: 1) SPTB c.647G>A, leading to spiculopathy, was identified in 8 patients of 2 unrelated families, 2) SPTA1 c460_462dupTTG, leading to ellyptocytosis, was identified in 6 patients from 5 different unrelated families.

Table 1. Baseline characteristics and genotypes of PK deficient patients

<table>
<thead>
<tr>
<th>Patient</th>
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<th>Hb</th>
<th>PK activity</th>
<th>ATP (ng/ml)</th>
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Summary/Conclusions: These data support the hypothesis that drug interaction with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of PKLR genotypes. The combination of these effects suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attractive way to correct the underlying pathologies of PK deficiency.

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IDENTIFICATION OF NEW PATHOGENIC MUTATIONS IN PATIENTS WITH RED BLOOD CELL MEMBRANE DISORDERS USING NEXT-GENERATION SEQUENCING

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Background: Red blood cell (RBC) membrane proteins deficiency or structural alterations lead to RBC membrane disorders such as hereditary spherocytosis, hereditary elliptocytosis or hereditary xerocytosis among others. Genetic analysis of these patients was not usually performed before next-generation sequencing (NGS) PANCAP test, a task 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 Lorrca) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 25 μM or 50 μM (37°C) prior to test. Baseline protein levels of PK-R were assessed using antibodies against PK-R.

Results: Baseline protein level analyses suggest strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Distinct metabolic changes were consistent with a block of glycolysis at PK-R step. Treatment of PK-deficient RBCs with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (mean increase 1.4-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 1.4-fold, range 1.2-2.2) similarly to control cells (mean increase 1.6 fold, range 1.4-1.8). Generally, PK-R residual activity and response to ellyptocytosis, was identified in 6 patients from 5 different unrelated families. The following therapeutics were used in 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 worth noting that almost 10 of the 13 undiagnosed patients had been oriented as unclear membraneopathy.

Conclusions: According to the results, there is a high genetic heterogeneity in patients with RBC membrane disorders, as almost each family carries a unique mutation that is not observed in any other no related family. The present study reveals the usefulness of NGS panel, which allows the molecular diagnosis of almost the 90% of the patients and it would avoid misdiagnoses. The diagnosis could lead to splenectomy in cases of mild disease, but it didn't suggest any specific RBC membrane pathology.

P616

CLINICAL FOLLOW-UP OF 378 PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: PROGNOSTIC IMPACT OF HEMOGLOBIN LEVELS, AUTOANTIBODY CLASS, AND RETICULOCTYPENIA AT ONSET ON THE RELAPSE RISK AND OUTCOME

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Background: Autoimmune hemolytic anemia (AIHA) is greatly heterogeneous, from mild/compensated to life-threatening, due to autoantibody class/thermal amplitude, efficiency in activating complement, activity of the reticuloendothelial system, and efficacy of bone marrow compensatory response.

Aims: Here we analysed predictors of first relapse, complications, and fatality in a large AIHA series.

Methods: We retrospectively studied 378 patients (135m and 243 F, median age 61 yrs, range 19-100) from 15 sites, followed-up for 0.5-27 years. Cases were classified in warm (w)AIHA (DAT positive for IgG and IgG+C), cold agglutinin disease, CAD (C), mixed (IgG+C with high titer cold agglutinins) and atypical (DAT-, IgA+, wIgM). Cases were also grouped in very severe (Hb<6 g/dl), severe (Hb 6-8 g/dl), moderate (Hb 8-10 g/dl) and mild (Hb>10 g/dl). LDH was expressed as fold increase upper the limit of normality (ULN), and reticulocytes as absolute count and reticulocyte index. The following therapy lines were considered a) steroids +/-IVg, b) rituximab c) splenectomy, d) immunosuppressive drugs (azathioprine, cyclophosphamide, cyclosporin), and e) transfusions, plasma exchange, erythropoietin.

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Summary/Conclusions: These data support the hypothesis that drug interaction with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of PKLR genotypes. The combination of these effects suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attractive way to correct the underlying pathologies of PK deficiency.
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<0.01), LDL higher in IgG+C wAIHA, mixed and atypical forms (p=0.01), and Hb and LDL values were negatively correlated (r=−0.25, p=0.001). Absolute reticulocytes were reduced in CAD, mixed and IgG+C wAIHA (p<0.01) 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), 2.9 (1.4-6.2), 3.4 (1.6-7.5), for Hb < 6, 6-8, and 8-10 g/dL compared to patients with Hb >10, respectively. As regards complications, infections were observed in 14% of cases, mostly mixed AIHA (p=0.02); thrombosis occurred in 10% and acute renal failure in 3% of patients, with no relationship with AIHA type/Hb values. Evans’ syndrome was more frequent in mixed or atypical cases (p=0.04) and in severe forms (74% with Hb<8 g/dL vs 26%, p=0.005), and was associated with higher relapse risk (HR 2.3, 95% CI 1.4-3.9). Seventy patients died during the follow-up, and 12 because of AIHA-related acute complications. Higher mortality was observed for infections (HR 5.8, 95% CI), acute renal failure (HR 7.6, 95% CI) and Evans’ syndrome (HR 8.3, 95% CI).

Summary/Conclusions: In conclusion, we found that severity of anemia at onset was the major determinant of relapse risk. The lowest Hb levels were observed in patients with IgG+C wAIHA and atypical cases along with higher LDL levels and inadequate reticulocytosis, advising strict clinical observation in these patients.

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HEME BINDS ANNEXIN-A5 DURING HEMOLYSIS AND PREVENTS ITS INTERACTION WITH CELL MEMBRANE PHOSPHATIDYLSERINE DURING SICKLE CELL DISEASE
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Background: Intravascular hemolysis, such as in sickle cell disease (SCD), is characterized by damage to red blood cells, high levels of cell-free heme and extracellular vesicles in plasma, along with inflammation and tissue injury. Stressed leukocytes, platelets, endothelial and red blood cells shed microparticles (MP) that bear externalized phosphatidylserine (PS) at their surface and promote tissue injury. Conversely, intracellular annexin-A5 acts as an inhibitor of externalization of PS at the surface of cells and MP. Annexin-A5 is 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 annexin, and annexin-A5 activity in particular, is blocked by intracellular heme as it is released in plasma during intravascular hemolysis.

Methods: In order to test the heme-annexin-A5 relationship, we measured PS+, PS+, CD235a+ and annexin-A5+ circulating MP in adult SCD patient and matched control plasmas. We explored annexin-A5 expression in plasma and blood cells by Western blots and ELISA, and also quantified the PS-binding functionality of plasma annexin-A5 using a self-designed immunocapture assay and purified PS+ MP. Moreover, we investigated molecular interactions between purified heme and recombinant human annexin-A5 by surface plasmon resonance (Biacore and Proteon), absorbance shift assay and protein autofluorescence (Proteon). 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 plasmon surface resonance assays, with human serum albumin and hemopexin in competition. The binding of annexin-A5 to heme-bound PS+ MP was further strengthened by the addition, 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 binds several sites for heme binding, some with very low affinity, while others are estimated with a Kd in the 10−6 m 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 the heme may bind to the heme-binding domain 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.
enizing anemia) considered related to study drug were reported in 2/10 patients. Increases in mean hemoglobin were anticipated due to the oncotic effects of this colloidal drug, but with no consistent pattern to the changes. Changes in ECG intervals were seen in a few subjects, but those changes were not considered clinically meaningful. There were no clinically meaningful changes in laboratory values, physical examinations, or concomitant medications. There were no statistically significant changes from Baseline in leg ulcer pain and wound surface area for either Cohort. All of the wound assessments remained relatively consistent throughout the study. There were slight decreases in total VCSS at most time points, indicating slight improvement in vascular status. Results were similar for the individual scores.

Summary/Conclusions: The administration of 4 or 6 once-weekly infusions of PEG-COHb at a dose of 320mg/kg was generally well tolerated. Slight improvements in total and individual VCSS are promising and may warrant further study with prolonged repeated doses of PEG-COHb.

P619
NON-RENAL DETERMINANTS OF ENDOGENOUS ERYTHROPOIETIN LEVELS IN SICKLE CELL DISEASE
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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 anaemia, age, sex, eGFR, white cell count, and use of hydroxycarbamide. Multivariate analysis revealed alpha globin status, Hb, CRP and HbF were elevated. Unlike the non-sickle setting where Ln EPO is very strongly positive correlation with Ln CRP, LDH, STFR, Ln uACR, cystatin C. One way ANOVA showed alpha globin status to be associated with EPO (higher EPO with more alpha chains). There was no significant association between EPO and: negative correlation with PCV, oxygen saturations, Ln HbF; and positive correlation with Ln CRP, LDH, STFR, Ln uACR, cystatin C. One way ANOVA showed alpha globin status to be associated with EPO (higher EPO with more alpha chains). There was no significant association between EPO and: age, sex, eGFR, white cell count, and use of hydroxycarbamide. Multivariate linear regression (N=175) revealed alpha globin status, Hb, CRP and HbF. Our findings suggest that in addition to Hb, other SCD severity markers influence EPO production. This may provide explanation for relative EPO deficiency, and have implications for considering therapeutic EPO in SCD.

P620
THE PHARMACOKINETICS (PK) OF GBT440 ARE SIMILAR IN ADOLESCENTS AND ADULTS WITH SICKLE CELL DISEASE (SCD)
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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 β0-thalassemia). Participants were enrolled after obtaining written informed consent/assent. This study is being conducted in 2 parts: Part A, single-dose, and Part B, multiple-dose for 24 weeks. The primary objective of Part A is PK and the primary objectives of Part B are safety and efficacy. PK samples to measure whole blood and plasma GBT440 concentrations were collected up to 15 days following single dose administration. Separate PPK models were developed to describe the concentration versus time profiles of GBT440 in whole blood and plasma using non-linear mixed effects modeling (NONMEM, version 7.3). The allometrically scaled adolescent PPK model was also used to estimate the appropriate single dose for subsequent evaluation in pediatric participants (6 to 12 years).

Results: Part A has been completed; 7 adolescents (3 males/4 females) received a single dose of GBT440 600mg. The median age of participants was 16 years (range 14 to 16 years) and the mean weight was 52.6 kg (range 44.6 to 65.8kg). GBT440 was well tolerated; there were no drug-related Grade 3 adverse events (AE) or serious adverse events and the most common AE was Grade 1 nausea. A 2-compartment model with first order absorption best described the PK of GBT440 and is the same model structure as previously used for adults with SCD. GBT440 PK parameters (Table 1) are comparable to those derived in adults, suggesting that GBT440 PK in adolescents and adults are similar. Model validation confirmed this result with good agreement between the observed adolescent PK data and simulated profiles based on the adult GBT440 PK model.

Table 1. Multivariate analysis of EPO.

<table>
<thead>
<tr>
<th>Alpha globin category</th>
<th>R2 (%) (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>-188 (-231 to -106)</td>
<td>.003</td>
</tr>
<tr>
<td>B-beta</td>
<td>-117 (-209 to -26)</td>
<td>.014</td>
</tr>
</tbody>
</table>

where alpha= 0 if aa/aa; 1 if aα/aα; 2 aα-aα

Summary/Conclusions: This is the first study used to develop a GBT440 PK 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., AUG) 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 proliferation of host cells and is also a biomarker for CD8+ cytotoxic T cells (CTLs). We previously analyzed the Tax-specific T-cell receptor (TCR) repertoire, phenotypes and functions of Tax-specific CTLs at the single-cell level in HLA-A24+ ATL patients who underwent allogeneic stem cell transplantation (allo-SCT). We found that a particular amino acid change in the TCR-PF (PF in the CDR3 region of TCR-β was conserved in different patients and also within the same patient before and after allo-SCT, and the PDR+ Tax-specific CTL clone selectively expanded in ATL long-term survivors as less-differentiated effector memory CTLs. Actually, the PDR+ CTL showed not only strong binding activity for the Tax-tetramer but also strong killing activity against patients’ HTLV-1-infected T-cells without any reaction against normal cells.

Methods: 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 effi cience against HTLV-1-infected T-cells and ATL-cells both in vitro and in vivo mouse model.

Results: Methods: HLA-A24-02 restricted and Tax301-309-specific TCR-β/γ genes were cloned from an established PDR+ CTL clone and integrated into a retroviral vector (Tax-siTCR vector) encoding small-interfering RNAs (siRNAs) to knockdown endogenous TCR genes for the efficient expression of therapeutic TCRs. Then, CD8+ T-cells of healthy volunteers were transfected with Tax-siTCR vector (Tax-siTCRs). First, cytotoxicity and cytokine production capability of the Tax-siTCRs against HTLV-1 infected T-cells or ATL-cells were evaluated using calcein-AM-based assay and flow-cytometric analysis, respectively. Next, to evaluate the in vivo anti-ATL effects by the Tax-siTCRs, the bioluminescence assay (in vivo imaging system) was performed. We generated a luciferase-gene transduced HLA-A24+HTLV-1 infected cell-line, MT-2 (Luc-MT-2), and injected 1x10^6 Luc-MT-2 cells into six-week-old NOD/Shi-scid, IL-2RγKO (NOS) mice intraperitoneally. After the 3 weeks, 2x10^6 Tax-siTCRs were administered, for a total of 3 times. Then, CD8+ T-cells (Mock) were administered in the same way. These mice were monitored for tumor growth using ISIV system weekly.

Results: Tax-siTCRs showed specific and strong killing activity against both HTLV-1 infected T-cells and patients’ ATL-cells without any reaction against control normal-cells. In addition, Tax-siTCRs 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-siTCRs had started to reduce gradually after 7 weeks, and finally became undetectable after 9 weeks. In addition, macroscopic anatomical findings in the treated mice were normal after 12 weeks. In contrast, the amount of bioluminescence in the mice treated with Mock or in the control mice without treatment had rapidly increased and all mice died by 9 weeks.

Summary/Conclusions: We confirmed that Tax-siTCRs 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-siTCRs has a potential to be a novel immunotherapy for ATL patients.

P622

Abstract withdrawn.

P623

NHEJ-BASED GENE EDITING: A NOVEL GENE THERAPY APPROACH IN FANCONI ANEMIA HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Background: Fanconi anemia (FA) is an inherited bone marrow failure syndrome caused by altered FA core complex (FACC) function, typically characterized by a defect in the non-homologous end joining (NHEJ) pathway. In FA patients, there is a higher incidence of subsequent cancer, and the limited availability of matched donors hamper the application of this therapy in FA patients. For this reason correction of patients’ HSPCs by gene therapy is considered a promising therapeutic alternative for these patients. In this context, gene editing constitutes a new step in the development of safe gene therapy approaches. Since non-homologous end joining (NHEJ) is the preferred DNA repair mechanism in HSPCs, and given that the NHEJ mechanism has been shown to be activated in FA cells, we have tested the efficiency of a NHEJ-mediated gene editing approach to generate compensatory mutations that can restore the FA gene function in HSPCs from FA patients, mimicking reversions observed in mosaic patients.

Aims: To demonstrate the feasibility of using a NHEJ-based gene editing strategy to correct FA-A HSPCs as a result of the insertions and deletions (INDels) generated in edited FA sequence in these cells.

Methods: Two different FA mutations from FA-A patient-derived lymphoblastic cell lines (LCLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDels generated in a as a consequence of the NHEJ were analyzed at different time points.

Results: Initial studies conducted in a FA-A LCLs carrying the bi-allelic c.295C>G point mutation that creates a premature stop codon (p.Q98X) showed targeting efficiencies around 20%. Next Generation Sequencing (NGS) not only revealed the presence of multiple repairing events, but also that these events were located downstream of the targeted site and not present in the edited cells. Moreover, reversion of the characteristic MMC hypersensitivity and restoration of the FANC2 foci formation were observed in these cells. In addition, western-blot analysis confirmed the stable expression of FA protein.

Summary/Conclusions: Our results demonstrate the feasibility of the approach, a second FA mutation (c.295C>G) was targeted, conferring a frameshift and a premature stop codon (p.R1187fsX28) with even higher gene targeting efficiencies. Finally similar studies were conducted in three HSPCs samples from FA-A patients harboring the c.295C>G mutation, that showed targeting efficiencies up to 36%. Moreover, NGS detected the presence of corrective NHEJ repair events immediately after editing and even 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.

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 immunomodulatory 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 this mechanism has been reported to be enhanced in FA cells, we have conducted initial studies in FA-A LCLs carrying the biallelic c.295C>T point mutation that creates a premature stop codon -p.R1187EfsX28-) with even higher gene targeting efficiencies. Finally similar studies were conducted in three HSPCs samples from FA-A patients harboring the c.295C>G mutation, that showed targeting efficiencies up to 36%. Moreover, NGS detected the presence of corrective NHEJ repair events immediately after editing and even 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.

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Aims: To demonstrate the feasibility of using a NHEJ-based gene editing strategy to correct FA-A HSPCs as a result of the insertions and deletions (INDels) generated in edited FA sequence in these cells.
phoma patients. The manufacturing process consistently allows high CAR transduction efficiency and expression of CD19 and T cells (75.31%±4.294 and 76.95%±14.76 respectively, n=8) and ensures the preservation of CD4−/INKT 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 CD8+ cytotoxic activity, and exert additive dual-specific cytotoxicity against CD19+ and CD19+ targets. Additional functional dissection showed that activated CARiNKT19 cells, both fresh and cryopreserved, have the ability to produce cytotoxic granules and IFNγ faster and in larger amounts than same donor activated CART19 cells. Likewise, CAR2- and CAR3-INKT cells are equally or more effective than their CART counterparts for killing CD19+ and lymphoid and non-lymphoid cell lines (B-lymphoblastoid C1RCd1 and lymphoma-derived Fargeau 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=0.23), both CART19 and CARiNKT19 cell-treated animals had significantly and comparably improved overall survival (P<0.001). However, compared to CART19, CARiNKT19 immunotherapy led to a better disease control, with earlier, more profound and sustained responses resulting in a significantly improved tumour free-survival (P=0.03).

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 INKT cells in protection from a gVHD supports the development of CARiNKT19 cells for ‘off-the-shelf’ use.

P625
A NOVEL CHIMERIC ANTIGEN RECEPTOR ENDOWS T CELLS WITH NK CELL-LIKE SPECIFICITY AND ATTACKS A WIDE RANGE OF HEMATOLOGICAL MALIGNANCIES AND CANCERS
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Background: Engineered T-cells expressing CD19-specific chimeric antigen receptors (CARs) have shown high response rates against relapsed and refractory B cell acute lymphoid leukemia (ALL). However, similar success has not yet been demonstrated in solid tumors, and the reasons for this are currently being investigated. One major obstacle is the difficulty in determining appropriate surface antigens that are effectively targeted by CAR-transduced immune cells. NKp44 is an activating receptor on human NK cells that is only expressed when the NK cells are activated, and which confers a marked increase in cytotoxic activity against various tumors. Ligands for NKp44 have been reported to be expressed in various types of cancers, but not in healthy cells. Effective use of the ligand-binding domain of this receptor as an antigen recognition site of a CAR would thus allow a wide range of cancer cells to be attacked.

Aims: To determine the optimal CAR construct including the NKp44 immunoglobulin domain as a ligand-binding domain (NKp44-based CAR), with a view to developing effective CAR-T therapy against hematological malignancies and solid cancers.

Methods: We created several NKp44-based CAR constructs. Human T cells from healthy donors were stimulated with anti-CD3/CD28 beads and 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 pattern 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, S-OS, S-OS-2, SaOS-2, U2OS,mg-63), rhabdomyosarcoma (RMS-YM, Rh28), and neuroblastoma (NB-1, NB-16). 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, CD8a, CD28, or DC3γ. A combination of the hinge domain from NKp44, transmembrane domain from CD28, and intracellular signaling domain from CD3ζ yielded the highest surface expression of CAR on both T cells and NK cells. T cells transduced with this CAR showed enhanced cytotoxicity against various target cells including AML, T-cell ALL, and B-cell ALL, but did not attack normal T cells. CAR-T cells also showed increased production of interferon-γ and granzyme B. The hinge domain from NKp44 significantly reduced cytotoxic function, though CAR expression levels remained similar.

Summary/Conclusions: T cells transduced with NKp44-based CARs show enhanced 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 REDIRECTED HUMAN T LYMPHOCYTES INDUCE POTENT ANTITUMOR IMMUNITY TO LEUKEMIA CELL LINES AND PATIENT-DERIVED ACUTE MYELOID LEUKEMIA IN NSG XENOGRAFT MODELS
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2German Cancer Consortium (DKTK), partner site Frankfurt / Mainz, Mainz, Germany

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 CAR expressing Tlymphocytes to combat CD19+ lymphomas have revealed compelling results. However, suitable antigens for an effective and specific CAR-mediated therapy to acute myeloid leukemia (AML) are still warranted as e.g. CD33 and CD123 CAR expressing T cells induce potent immune responses to leukemia lines and primary AML blasts in vitro and in vivo using NSG xenograft mouse models following ACT.

Methods: PBMCs or MACS® purified human T cells were polyclonally stimulated and reprogrammed with a CAR composed of the extracellular region of the NKp30 receptor fused to the CD3ζ chain signaling domain (kindly provided by Dr. S. Klobuch, Dept. of Internal Medicine 3, Medical University Regensburg, Germany) by retroviral gene transfer. Transduced T cells were further selectively expanded utilizing puromycin resistance present on the retroviral backbone, and NKp30 expression was determined by flow cytometry. IFN-γ ELISPOT analyses and cytotoxicity assays were performed to assess antileukemic responses to leukemia lines and primary AML blasts in vitro and in vivo using NSG xenografts and adoptive transfer of redirected T cells. Expression of CD7BH in target cells was confirmed by RNA-based RT PCR.

Results: Following transduction and puromycin selection ≥80% of CD3+ T cells expressed the NKp30 CAR. In addition, most T cells displayed an effector-mem- rory phenotype. Upon adoptive transfer the with the B7H6 expressing targets such as K562 and HL-60 (myelogenous leukemia cell lines), NALM 16 (pre-B-ALL) and patient-derived AML samples (e.g. M2Z06 and M2Z87) NKp30-redirected T cells elicited potent IFNγ release and exhibited cytolytic activity to both leukemia lines and primary AML blasts in vitro. These responses were specific as e.g. no reactivity to B7H6 negative myelogenous leukemia line U266 was observed. We then evaluated antitumoral responses of NKp30-redirected T cells in vivo. Upon adoptive transfer of NKp30-CAR T cells into NSG mice engrafted with K562 significant reduction of tumor burden was observed. Moreover, injection of 1 - 5×106 HLA-A2+ AML cell line 7BH6+CD33+CD123+ T cells into NSG mice showed up to 5% engraftment of patient derived AML blasts and thus resembling a clinically relevant minimal residual disease status at time of ACT resulted in clear leukemia regression. Further experiments e.g. to elaborate to what extent CD4+ and CD8+ T cells contribute to this antileukemic immunity are in progress.

Summary/Conclusions: These studies demonstrate that human T lymphocytes can be successfully redirected to acute leukemia by NK cell activating receptor based CARs such as the NKp30-CAR. As its ligand B7H6 has not been reported to be expressed on CD34+ HSC, this antigen might be an interesting target for adoptive immunotherapy to AML.

P627
PRECLINICAL TESTING OF ADOPTIVE T-CELL RECEPTOR GENE TRANSFORMATION IN COMBINATION WITH COMPLEMENTOINHIBITORS AS A NOVEL THERAPY FOR MULTIPLE MYELOMA
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Background: Adoptive cellular therapy (ACT) based on T-cell receptors (TCR) or chimeric antigen receptor (CAR)-engineered T-cells has achieved tremendous success in the treatment of cancer, especially B-cell malignancies. The
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 underlines 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 T-cell driven antitumor effect of adoptively transferred antigen-specific T cells. We propose to target multiple myeloma (MM) tumor cells in our established xenograft in vivo adoptive cell therapy model by T cells equipped with two optimized TCRs specific for HLA-A2.1-restricted MDM2 and p53 epitopes in combination with checkpoint inhibitors.

**Methods:** Human T cells from healthy donors were retrovirally transduced with MDM2- and p53-specific TCRs and expression levels were analyzed by flow cytometry. MDM2 and p53 protein expression in MM cell lines was determined by Western blot. The therapeutic efficacy of adoptive TCR transfer was evaluated in NOD-scid IL2R γ chainnull (NSG) mice engrafted (s.c) with HLA-A2.1-expressing MM cell lines. In the establishment of adoptive TCR transfer, mice were transplanted with adoptively transferred NOD-SCID IL2R γ chainnull (NSG) mice. 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 treatment, demonstrating the need to target multiple MM antigens to circumvent tumor escape mechanisms associated with down-regulation of antigen. Yet, we observed a strong up-regulation of PD-L1 expression in tumor cells in vivo and a significant increase in TILs in NSG mice. The expression of PD-L1 and the administration checkpoint inhibitors (anti-CTLA-4 or anti-PD-1 antibodies) results in an increase of specific TILs 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 eradication suggesting that targeting one single immune checkpoint receptor is not sufficient for robust and durable tumor responses.

**Summary/Conclusions:** Combination checkpoint inhibitor approach has demonstrated significant potential in our ACT experimental MM model and forms the basis for a novel multi-modal immunotherapeutic combination treatment for multiple myeloma.

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**P629**

**EXPLORING HUMAN TCR- AND CAR-REDIRECTED INKt CELLS FOR ADOPTIVE CELLULAR THERAPY**

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**Background:** T cell receptor (TCR) - or chimeric antigen receptor (CAR) redirected T cells have substantially improved adoptive cellular therapy (ACT) for hematological malignancies and solid tumors. However, severe toxicity and 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 a significant increase in TILs in NSG mice. The expression of PD-L1 and the administration checkpoint inhibitors (anti-CTLA-4 or anti-PD-1 antibodies) results in an increase of specific TILs 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 eradication suggesting that targeting one single immune checkpoint receptor is not sufficient for robust and durable tumor responses.

**Aims:** In this current project of concept study we therefore explored human, AML-reactive TCR- and CD19 CAR-redirected INKt cells for their potential to induce antitumoral responses to leukemia cell lines as well as patient derived, primary AML blasts.

**Methods:** INKt cells expressing the invariant TCR composed of the Vα24Jα18/Vβ11 chains were immuno-magnetically isolated from PBMC derived from adult healthy donors using Vβ11-Ab (6B11)-conjugated, anti-iNKT microbeads (Miltenyi Biotec) and expanded in vitro upon coculture with autologous dendritic cell (DC) infected with anti-CD152 (CTLA-4) or anti-CD137 (4-1BB) agonist low amounts of interleukin (IL)-2. INKt cells were retrovirally transduced on day 6 after stimulation and selected for TCR or CAR expression utilizing a virally transduced puromycin resistance. While phenotypic analyses on INKt markers and on the percentage of redirected cells were performed by flow cytometry functional assays were performed. Notably, we observed functional heterogeneity and advanced differentiation of ex vivo expanded redirected lymphocytes limits their therapeutic potential and consistent efficacy. Invariant (type I) natural killer T (iNKt) cells have been demonstrated not only to promote effector functions of dendritic cells (DC), natural killer (NK) cells and T cells but also to localize to tumors and have inherent antitumoral properties. Moreover, as these cells are further restricted to the monomorphic, HLA class I-like CD1 molecule expressed only on a few cell types with limited alloreactive potential, all these features make INKt cells as attractive alternative carriers for redirected therapy.

**Results:** Following isolation of 0.7 - 0.8 × 10^6 Vs24Jα18/Vβ11+ INKt cells from PBMC we achieved on average a 120-fold expansion 21-28 days after stimulation with GaICer loaded, irradiated autologous DC and 25 dB IL-2. Additional use of lenalidomide to promote expansion as described previously had no effect. Expanded INKt cells were mainly CD4* (83%) and about 80% of cells expressed the natural killer receptor CD161 described as iNKt maturation marker but showed limited or virtually no expression of typical NK markers CD56 and CD16. Following retroviral transduction and selection for 6 days >80% of TCR (5B2) - and CD19 CAR-redirected INKt cells were demonstrated. Subsequent functional analyses revealed that both INKt cells expressing the AML-reactive TCR 5B2 as well as CD19-CAR iNKt cells demonstrated substantial release of IFN-γ and elicited potent antileukemic responses to AML cell lines as well as patient derived, primary AML blasts.

**Summary/Conclusions:** These studies demonstrate that purified human Vs24Jα18/Vβ11+ INKt cells expanded from PBMC can be successfully redirected against acute leukaemia both by TCR and CAR expression. Engineered INKt cells might therefore be promising alternative carriers for redirected ACT or being used in combination with redirected T cells as combined immunotherapy.

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**P630**

**SPECIFIC TARGETING OF ACUTE MYELOID LEUKEMIA BY THE USE OF A Chimeric Antigen RECEPTOR (CAR) TO EXPRESS THE ANTI-CD33 CHIMERIC ANTIGEN RECEPTOR (CAR)**

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**Background:** Acute myeloid leukemia (AML) is the most common subtype of acute leukemia both in children and adults. Despite recent improvements, AML is still often observed either transiently or in a minority of patients. This underlines 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 T-cell driven antitumor effect of adoptively transferred antigen-specific T cells. We also characterized the expression of BAFF-R molecule, six anti-BAFFR CAR genes that differ for the inversion of the VH and VL and the length of the spacer domain that have been generated. Cytokine-induced Killer (CIK) cells, engineered using an improved Sleeping Beauty (SB) transposon system, stably expressed anti-BAFF- CARs, and maintained their characteristic phenotype. Among the newly constructed CARs, the shortest VHV anti-BAFFR CAR exerted the highest anti-leukemic activity towards target cells, such as NALM-6, with an in vitro killing efficiency of up to 90%.

**Methods:** We developed six anti-BAFFR.CARs that differ for the inversion of the VH and VL and the length of the spacer domain (CAR) approach targeting BAFF-R molecule, six anti-BAFFR CAR genes that were engineered using an improved Sleeping Beauty (SB) transposon system, stably expressed anti-BAFF-CARs, and maintained their characteristic phenotype. Among the newly constructed CARs, the shortest VHV anti-BAFFR CAR exerted the highest anti-leukemic activity towards target cells, such as NALM-6, with an in vitro killing efficiency of up to 90%. We also detected a specific cytokotoxic activity towards primary B-ALL blasts (average 65±4,5%, n=9). Combining the INVsh.CAR with CD19.CAR we detected a superior antitumor activity towards ALL targets (average 72,2±22,5%, n=9). In vivo assays revealed that INVsh.CAR redirected primary 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 lyse CD19-negative blasts.

**Summary/Conclusions:** Taken together, these findings make this receptor a relevant and tractable target to develop second line B-ALL immunotherapy in case of relapse after CD19-targeting therapies or for a double targeted approach. Being restricted to mature B cells, but absent on precursors and plasmablasts, our strategy could have an inferior toxicity concerning the emergence of B-cell aplasia observed in patients treated with anti-CD19 CAR-modified T cells.
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 treatment options, interest has grown in the use of 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 acute myeloid leukemia (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 Cytoxic Induced Killer (CIK) cells alone and in combination with standard chemotherapeutic agents.

**Methods:** We proved the feasibility of harnessing Cytoxine Induced Killer (CIK) cells as a third generation anti-CD33 CAR through the non-viral Sleeping-Beauty transposon system, starting from fresh and frozen healthy mononuclear cells (PBMCs) and also from frozen primary AML samples. The in vitro anti-AML activity of CD33.CAR-CIK cells is assessed by means of cytotoxicity, proliferation and cytokine production assays upon challenge with AML cell lines and primary samples. The in vivo efficacy of CD33.CAR CIK cells is evaluated in NSG mice transplanted with AML cell lines (M2a-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 (Ara-C and doxorubicin).

**Results:** CD33.CAR-CIK cells were able to induce a potent anti-leukemic activity as compared to unmanipulated CIK cells, in terms of specific killing (up to 70%), proliferation (up to 40% of Ki67+CAR-CIK cells) and cytokine production (up to 30% for both IL-2 and IFN-gamma producing CAR-CIK cells) when challenged with both AML cell lines and primary leukemic cells. By treating M2a-NRas cell grafted mice with the already established 5+3 induction chemotherapy protocol, we confirmed that chemotherapy is able to significantly reduce the leukemic burden from around 20% to 0.1% in the bone marrow. Since the AML disease is not totally eradicated, this model will be therefore suitable to further investigate the efficacy of the CD33.CAR.CIK cells immunotherapy on the chemotherapy resistant/residual AML cells.

**Summary/Conclusions:** Having demonstrated the significant in vitro anti-leukemic activity of SB-modified CD33.CAR-CIK cells we next aim to assess their efficacy in vivo, particularly against the resistant/residual AML cells that were not eradicated by standard chemotherapy treatment. Moreover, envisaging a safer clinical translation of this immunotherapeutic approach, a transient CAR expression, by using CD33.CAR coding mRNA, is under investigation, in order to limit the potential myelotoxicity due to the long-term off-target effect on normal hematopoietic stem/myeloid progenitor cells. Finally, if successful, our results will provide the preclinical validation of CD33.CAR-CIK cell immunotherapy, supporting its development to the clinic.

**P631 UPDATE ON THE FIRST PATIENTS WITH SEVERE HEMOGLOBINOPATHIES TREATED WITH LENTIGLOBIN GENE THERAPY**

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**Background:** Insertion of an anti-sickling β-globin gene variant into hematopoietic stem cells (HSCs) could reduce or eliminate symptoms of severe sickle cell disease (SCD) and transfusion requirements in transfusion-dependent β-thalassemia (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 (ΔAT87Q) designed to confer anti-sickling properties similar to γ-globin. We recently (ASH 2016) reported 23 months of follow-up for a patient with SCD, and 12–34 months of follow-up for 4 patients with TDT.

**Aims:** To evaluate the safety and efficacy of LentiGlobin gene therapy for severe hemoglobinopathies.

**Methods:** Patients 5-35 years old with severe SCD (e.g., ≥2 acute chest syndrome episodes or ≥2 vaso-occlusive crises [VOC] in the preceding year) or TDT (≥100 mL/kg of packed red blood cells [PRBC] per year) were enrolled. After informed consent, autologous CD34+ cells were collected and transduced with the BB305 vector. Patients underwent myeloablative conditioning with busulfan prior to infusion of transduced cells. Patients were then monitored for hematologic engraftment, vector copy number (VCN), genetically engineered hemoglobin (HbAT87Q) levels, and adverse events (AEs). Disease-specific assessments included transfusion requirements for TDT, or VOCs and hospitalizations for SCD.

**Results:** As of September 2016, 1 patient with severe SCD (male; 13 years old) and 4 patients with TDT (2 male, 2 female; 16–19 years old) have received LentiGlobin DP in Study HGB-205. The median DP cell dose was 8.9 (range 5.6-13.6) x106 CD34+ cells/kg with a DP VCN of 1.2 (range 0.8–2.1) vector copies/diploid genome. Median post-infusion follow-up was 22.9 months (range 11.6-33.5). All subjects engrafted successfully with median time to neutrophil engraftment of 17 (range 14-38) days. Within patients, VCN in peripheral blood remained generally consistent from Month 3 (range 0.3–3.3 at last measurement). The toxicity profile was consistent with myeloblastic conditioning with single-agent busulfan, with no ≥Grade 3 DP-related AEs or serious AEs and no evidence of clonal dominance reported to date. The patient with severe SCD who, prior to study enrollment, received regular RBC transfusions, experienced no clinical symptoms or complications of SCD in the 21 months since treatment. At Month 21, his total Hb was 13.1 g/dL, with 6.2 g/dL HbAT87Q (48%) and 6.5 g/dL sickle Hb (HbS: 50%); in addition, their unconjugated bilirubin, lactate dehydrogenase and reticulocyte count had dropped by 56%, 58%, 26%, respectively, compared to screening. Of the 4 patients with TDT, 3 have β0/βE genotypes and 1 is homozygous for a severe β+ mutation (IVS1 nt 110 G>A). Two of the β0/βE patients have completed their 2-year primary follow-up and entered a long-term follow-up study. They have been without RBC transfusions for 33 and 30 months, with total Hb of 10.9 and 13.5 g/dL, and HbAT87Q of 7.7 and 10.1 g/dL, respectively. The third patient with a β0/βE genotype has 12 months follow-up and has not required transfusions since 4 days post-LentiGlobin DP infusion, with total Hb 11.3 g/dL and HbAT87Q of 8.6 g/dL. The patient with the IVS1 genotype has 15 months of follow-up and has been free of transfusions for 11.6 months, with total Hb 8.3 g/dL and HbAT87Q of 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 HbAT87Q levels, which alleviate the clinical and biochemical effects of severe SCD and TDT, with safety consistent with myeloblastic conditioning. Follow-up data on the 5 previously reported patients and early results from the 2 recently treated patients will be presented.
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 lympho-proliferative 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 27.8%, and bone marrow involvement in 69.3%. 24.9% had autoimmune diseases and 9.2% had autoimmune cytopenias. 45.6% were observed while the remainder required at least one line of therapy. 5-year and 10-year OS were 75.0% and 63.1%, respectively. There was no statistically significant difference in OS, complete response rate or duration of response based on first line agent (methotrexate, cyclophosphamide, cyclosporine A). However, there was a statistically significant improved partial response with methotrexate versus other therapies (p<0.01). A marginally significant association between severe anemia/transfusion dependence and poor overall response rate (p=0.079) 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 the 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.

INDOLENT NON-HODGKIN LYMPHOMA - CLINICAL

ONGOING PHASE 1/2 STUDY OF INCBO50465, A SELECTIVE PI3K-DELTA INHIBITOR, FOR THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY B-CELL MALIGNANCIES (CITADEL-101)

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Background: Signaling networks mediated by PI3Ks have been implicated in proliferation, migration, and function of B-cells. INCBO50465 is a novel, potent, and selective inhibitor of PI3Kδ (≥19,000-fold more selective for PI3Kδ vs other isoforms). INCBO50465 demonstrated linear pharmacokinetics (PK) and achieved exposure levels several-fold greater than the 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 (NCT02018881).

Methods: In this phase 1/2 study, eligible patients ≥18 years of age had relapsed/refractory lymphoid B-cell malignancies (excluding Burkitt's lymphoma and precursor B-cell lymphoblastic leukemia/lymphoma), Eastern Cooperative Oncology Group performance status score ≤2 (≤1 during dose escalation), normal liver and kidney function, and had not received autologous hematopoietic stem-cell transplant (HSCT) within 3 months or allogeneic HSCT within 6 months of screening. The protocol was initiated with a single-patient cohort, treated with oral INCBO50465 5mg QD. Subsequent cohorts used a 3+3 design and evaluated doses of 10–45mg QD. Based on PK/pharmacodynamics, the 20 and 30mg QD cohorts were expanded. Responses were assessed every 9 weeks using the Lugano Classification or International Working Group on Chronic Lymphocytic Lymphoma (CLL) criteria.

Results: As of the data cutoff (Nov 1, 2016), 52 patients were treated (median age 65 years, range (35–88)). Baseline disease subtypes included diffuse large B-cell lymphoma (DLBCL; n=14), follicular lymphoma (FL; n=10), Hodgkin lymphoma (HL; n=9), marginal zone lymphoma (MZL; n=8), CLL (n=6), and mantle cell lymphoma (MCL; n=5). Sixty-two percent (n=32) of patients had ≥3 prior systemic regimens; 31% (n=16) had prior HSCT. Median duration of therapy was 14.7 months (range, 0.6–13.4); no DLTs were identified. Seventy-seven percent of patients discontinued therapy, most commonly due to disease progression (31%) and AEs (25%). Thirty-three percent of patients had dose interruption and 2% had dose reduction. Most common nonhematologic AEs (all grade: grade ≥3) were nausea (38%; 0%), diarrhea (31%; 6%), and vomiting (25%; 0%). Grade ≥3 hematologic AEs included neutropenia (21%), lymphopenia (17%), thrombocytopenia (10%); and anemia (4%). Forty percent of patients had serious AEs (SAEs), most frequently colitis, diarrhea, and hypotension (all n=3). One patient had grade 3 pneumonitis; none had Pneumocystis jirovecii pneumonia (PJP) or grade ≥2 elevated transaminase. Objective responses occurred at all doses (Table 1), except 5mg QD; 90% of the objective responses were observed at the 9-week disease assessment.

Summary/Conclusions: In patients with relapsed/refractory B-cell malignancies, INCBO50465 demonstrated manageable toxicities with no clinically meaningful transaminisits or PJP. Objective response rates were generally high and most responses (90%) were observed at the 9-week disease assessment. Different dosing regimens/schedules, long-term safety, and disease-specific cohorts are being evaluated.

PHASE IIIB RANDOMIZED STUDY OF LENALIDOMIDE PLUS RITUXIMAB (R2) FOLLOWED BY LENALIDOMIDE VERSUS 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) NHL show tolerability and high activity for the combination of lenalidomide plus rituximab (R2) and support further study of R2.

Aims: The current study assesses 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.
lymphoma (IFL). Upon informed consent, patients receive 12 cycles of R² induction (weekly vidarabine 20mg/m², 21 of 28 d; rituximab 375mg/m² weekly cycle 1 [d1, 8, 15, 22], then d1 of odd cycles). Responders to induction (≥SD) are randomized: 1:1 to maintenance with either R² or rituximab alone (18 cycles); following R² maintenance, optional single-agent lenalidomide (10mg/d, 21 of 28 d) can be given until PD. The primary endpoint is progression-free survival (PFS).

Results: As of April 14, 2016, 106 patients with R/R FL have been enrolled, including 103 with grade 1-3a FL, 2 with tFL, and 1 unknown grade. Median age of patients with FL was 66 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 (97%) patients had received prior rituximab-containing treatment, of which 35% were rituximab refractory (defined as best response of SD/PD to rituximab/rituximab-containing regimen or a CR/PR of <6 mo after the last rituximab dose). The most common prior regimens were rituximab alone (40%), R-CHOP/R-CHOP-like (38%), and bendamustine plus rituximab (35%). Premature discontinuation of lenalidomide occurred in 39 (37%) patients during the induction period, mainly due to AEs (n=20); the most common treatment-related AE leading to early discontinuation in the induction period was neutropenia in 8 patients. Four (4%) patients’ disease continued 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 (79% vs 55%; Table 1). The median time to response during induction was 2.8 mo. Twenty patients had 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.

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A DOUBLE-BLIND, RANDOMIZED PHASE 3 STUDY TO COMPARE EFFICACY AND SAFETY OF CT-P10 TO INNOVATOR RITUXIMAB IN COMBINATION WITH CVP IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED FOLLCULAR 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) (Coffleir, 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 CVP (cyclophosphamide, vincristine,

and prednisone) therapy every 3 weeks over 8 cycles. Overall response rate (ORR) was assessed on best overall response. Efficacy was assessed by the independent review committee, according to the 1999 International Working Group criteria.

Results: Therapeutic NI of CT-P10 to RTX has been demonstrated in terms of ORR over 8 cycles (Table 1). The ORR difference between two treatment groups was 4.3% in patients with positive anti-drug antibody 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).

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 CVP therapy in previously untreated AFL. CT-P10 was well-tolerated and the safety profile including immunogenicity of CT-P10 was comparable to that of RTX over 8 cycles in induction period.

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DURABLE DISEASE CONTROL OF EARLY MYCOSIS FUNGOIDES PATIENTS TREATED WITH LOW-DOSE INTERFERON-ALPHA2B AND PUVA

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Background: Early stage Mycosis Fungoides (MF) has an indolent, relapsing course, with patients frequently undergoing multiple therapies. Current guidelines consider the utility of combination therapies (skin-directed therapies plus systemic biologic response modifiers) to increase the therapeutic efficacy. Recently, time to next treatment (TTNT) was applied as a new relevant measure of the durability of response of PUVA, interferon-alpha (IFN-α) and retinoids as monotherapies in early MF (Hughes et al, Blood 2015; Hanel et al, AJH 2016), but it has not been yet investigated in combination therapies.

Aims: We aimed to evaluate TTNT together with the usual time-to-event measures (OS and EFS) in the series of 89 early MF patients treated for 14 months with interferon (IFN-α2b 6-18 MU weekly) and PUVA which was first described in 2005 (Rupoli et al, EJH 2005). The follow-up was prolonged up to October 2016, in order to evaluate prospectively the regimen activity and influence on the further course of the disease.

Methods: The design, rationale, safety and efficacy results for this protocol were previously published. Clinical studies 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 in 22 patients, IB in 55, IIA in 11, and IIB in 1 patient. The majority of patients had generalized skin disease (75% T2 vs 25% T1). The protocol proved to be highly effective, well tolerated and able to induce complete clearing of skin lesions in 84% of responders (88% of OS and 85% EFS). 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

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were 99%, 98%, 92%, 89%, 78% and 51%; at 1, 2, 5, 10, 15 and 20 years 98%, 97%, 88%, 67%, 19%, 0%, were free from events. Median TTNT was not reached thus indicating clinical benefit with IFN-α and PUVa. Kaplan-Meier estimated rates of 97% at 1 year, and 91% at 2 years, respectively whereas 5-, 10-, 20-year TTNT remained almost unchanged with 62% of patients that still had not required further treatment.

Summary/Conclusions: There has been an ongoing debate about whether patients would benefit from adding PUVa to IFN-α in the treatment of early stage MF. We chose to initiate the combination treatment of MF as early as possible in the course of the disease to induce a permanent remission or even a cure. In our experience, this regimen set the realistic goal of achieve high rates of complete clearing and durable responses (median TTNT not reached) with only 38% of patients requiring a subsequent systemic treatment within 20 years. Here, we suggest a synergistic or additive effect between PUVa and IFN-α compared with either agent alone. With respect to Hughes et al. (Blood 2015), our combination treatment provide a longer TTNT than PUVa or IFN-α monotherapy (36.3 months and 17.7 months respectively). At 2 years, 91% of patients receiving PUVa plus IFN-α were free from further treatment as compared to 54.2% and 29.1% treated with PUVa or IFN-α monotherapy, respectively.

P637

PHASE 3 ALCANZA STUDY: THE BRUTUXTUMAB VEDOTIN (BV) OR PHYSICIAN’S CHOICE (PC) OF METHOTREXATE (MTX) OR BEXAROTENE (BEX) IN CD30-POSITIVE CUTANEOUS T-CELL LYMPHOMA (CTCL): NUMBER NEEDED TO TREAT ANALYSIS

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Background: ALCANZA is a Phase 3 study of BV (Mtx or Bex) for the treatment of CD30-positive (CD30+) CTCL (NCT01578499). BV was associated with significantly improved rate of objective response lasting ≥4 months (ORR4; 56% vs 13%; p=0.0001), longer median progression-free survival (PFS; 16.7 vs 3.5 months; p=0.0001), and decreased symptom burden measured by SkinEx-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.

Methods: The ALCANZA data suggest that, at various time points, and dependent on risk group, the outcomes of patients with OAL in patients with diverse histologic subtypes. 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 and non-MALT lymphoma subtype (hazard ratio; HR=5.96, p<0.001 and HR=2.96, p=0.025, respectively), the risk factors-associated OS was only non-MALT lymphoma subtype (HR=9.18, p=0.013). Then, subgroup analysis

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 analysis</th>
<th>BV (n=64)</th>
<th>PC (n=64)</th>
<th>NNT</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>21</td>
<td>25.6</td>
<td>17.3</td>
<td>2.35</td>
<td>3.10, 1.7</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>23.2</td>
<td>17.3</td>
<td>1.14</td>
<td>1.40, 0.90</td>
</tr>
<tr>
<td>12</td>
<td>17</td>
<td>19.6</td>
<td>17.3</td>
<td>1.68</td>
<td>1.90, 1.50</td>
</tr>
<tr>
<td>18</td>
<td>9</td>
<td>18.3</td>
<td>17.3</td>
<td>2.19</td>
<td>2.40, 2.00</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>18.3</td>
<td>17.3</td>
<td>2.19</td>
<td>2.40, 2.00</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 and non-MALT lymphoma subtype (hazard ratio; HR=5.96, p<0.001 and HR=2.96, p=0.025, respectively), the risk factors-associated OS was only non-MALT lymphoma subtype (HR=9.18, p=0.013). Then, subgroup analysis
according to histopathologic subtypes, BM involvement alone was regarded as a statistically significant factor in the group of non-MALT lymphoma (HR=3.19, p=0.013) and there were no statistically significant factors in the group of non-MALT lymphoma. Although there were no risk factors with statistical significance, the BM involvement and advanced TNM stage showed a trend toward statistical significance about affecting to the failure of DFS (BM involvement of HR=1.90, p=0.054 and advanced TNM stage of HR 3.06, p=0.056). The median time-to-progression (TTP) was from 3 to 3.5 years after initial therapy in relapse or dead patients (range from 4.6 to 109.6 months).

Summary/Conclusions: Our study confirmed that OAL of all histologic subtypes also represented the indolent nature and localized behavior with favorable survival outcomes. Although BM involvement OAL consisted of a small number, it was associated with poor survival outcomes. Also, relapse and lymphoma-related mortality had long-term delayed TTP, so we suggested that BM biopsy might be a necessary study for initial staging at least in all OAL and long-term follow-up is required for patients with all histologic type of OAL.

P639

CLONAL B-CELL LYMPHOCYTOSE OF MARGINAL ZONE ORIGIN (CBL-MZ): A PROSPECTIVE REGISTRATION 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 recognized as a provisional entity in the WHO classification. Despite

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, MND, T-bet and IRTA-1. Gastroscopy with multiple biopsies was performed in 58 cases. FISH analysis for del7q was done in 13 pts, and detection for MYD88 mutation in 60.

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Case % (96 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>70 yrs</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 58 (60)</td>
</tr>
<tr>
<td>ALC (median)</td>
<td>4500/μL</td>
</tr>
<tr>
<td>Circulating B-cells (median)</td>
<td>2800/μL</td>
</tr>
<tr>
<td>β2-microglobulin (median)</td>
<td>2.6 mg/L</td>
</tr>
<tr>
<td>Serum creatinine (median)</td>
<td>0.9 mg/L</td>
</tr>
<tr>
<td>Protein (median)</td>
<td>5.9 g/dl</td>
</tr>
<tr>
<td>Alanine aminotransferase (median)</td>
<td>37 U/L</td>
</tr>
<tr>
<td>γ-glutamyl transferase (median)</td>
<td>18 U/L</td>
</tr>
<tr>
<td>Albumin (median)</td>
<td>4.1 g/dl</td>
</tr>
<tr>
<td>Platelet (median)</td>
<td>175 000/μL</td>
</tr>
<tr>
<td>Haemoglobin (median)</td>
<td>13 g/dl</td>
</tr>
</tbody>
</table>
| H.pylori (+) gastritis was evident in 30%. H. pylori gastritis was evident in 30%. H. pylori 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 MND 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, and 1 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 evolves 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.

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SAFETY OF SUBCUTANEOUS ADMINISTRATION OF RITUXIMAB DURING THE FIRST-LINE TREATMENT OF PATIENTS WITH NON-HODGKIN LYMPHOMA: THE MABRELLA STUDY

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Background: Intravenous (IV) rituximab is the mainstay of treatment for CD20+ B-cell non-Hodgkin lymphoma (NHL). A subcutaneous (SC) formulation of rituximab has been approved in Europe and other countries that reduces health-care resource burden and improves patient (pt) satisfaction and convenience compared with rituximab IV. MabRella is a global umbrella study comprising three local open-label, single-arm, Phase III studies of rituximab SC, which share a core protocol and primary endpoint but have flexibility for exploratory endpoints (NCT01889069; NCT01987505; NCT02406092). Data from participating countries are pooled for predefined global analyses.

Aims: To evaluate the safety of first-line (1L) rituximab SC in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) with a focus on administration-related reactions (ARRs).

Methods: Eligible pts were aged 18–80 years with grade 1–3a FL/DLBCL and ECOG performance status ≤3. All pts had received ≥1 full dose of rituximab IV. Only pts who switched to rituximab SC were included in this analysis. Updated data are presented (data cut-off February 7, 2017).

Table 1.

<table>
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<td>175 000/μL</td>
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<td>Haemoglobin (median)</td>
<td>13 g/dl</td>
</tr>
</tbody>
</table>
| Results: A symptomatic presentation of the main characteristics of CBL-MZ is given in the table. The median age was 70 yrs 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.

Results: The safety population comprised 421 pts: 160 Italy; 140 Spain; 121 North Africa (Tunisia, Morocco and Algeria). Median age was 58 years (range 19–80); 49% of pts were male; 225 pts had FL and 196 had DLBCL. Of the pts with FL, 97 completed ≥1 cycle of rituximab SC induction (45 completed 7 cycles) and 204 completed ≥1 cycle of maintenance (175 completed 6 cycles;
monitoring should be part of physician’s practice in these WM patients.

RF are reproduced in our real-life cohort despite older ages, and high IPSS to challenge standard R-based regimens with ibrutinib. Two parameters decreased the duration of PFS2 with immunochemotherapy: 47 DRC (PFS2 47mo), and 25 RF (PFS2 66mo), not significantly different. Only predicted PFS and OS with good accuracy. RF significantly increased the risk of Richter, and CLB exposure the risk of prognostication. Long-term follow-up: 22% of patients had second solid cancers.

symptomatic Waldenstrom macroglobulinemia (WM) may be for delayed toxicities (infections 39% and anemia<11.5g/dl decreased PFS. Previous CLB therapy increased the risk respectively. Dose reductions>20% had no impact on these outcomes, but age>65y potentially more long term side effects.

Results: 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 treatment completion (66/72 cases (91.7%) vs 59/66 cases (89.9%), 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.

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MICAVERSUS LIPSOSOMAL AMPHOTERICIN B FOR EMPIRICAL ANTFUNGAL THERAPY IN FEBRILE NEUTROPHIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED TRIAL

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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, while fever is an absolute indication to start broad-spectrum antibacterial 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 treatment completion (66/72 cases (91.7%) vs 59/66 cases (89.9%), 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.
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), anidulafungin (ANID), and isavuconazole (ISA), as well as the echinocandins caspofungin (CAS) and micafungin (MIC), and the polyenes amphotericin b (AmB) and liposomal amphotericin b (LAmB). Furthermore, PMN were simultaneously stimulated with lipopolysaccharides (LPS) or zymosan. Afterwards, PMN were analyzed by flow cytometry regarding activation, degranulation, and phagocytosis. Additionally, a dichotomous assay was used to detect reactive oxygen species (ROS). IL-8 synthesis was measured by enzyme-linked immunosorbent assay (ELISA). Secondly, a murine model was used to investigate the influence of MIC and POS on the clinical course of IPA in vivo. Therefore, mice were treated with antifungals and inoculated with Aspergillus fumigatus conidia. Afterwards, mice were analyzed concerning fungal burden and pulmonary damage (albumin ELISA) with neutropenic animals serving as controls.

Results: In vitro, pretreatment with POS lead to enhanced activation (CD62L: 44% ± 8 vs 13 ± 2, *; mean ± SEM, p value ≤0.05 considered to be significant). Fluconazole, and not voriconazole, and dosed generation of ROS (2688 rfu ± 4 to 5828 ± 161, *), whereas zymosan triggered IL-8 synthesis was reduced by trend. In contrast, ISA pretreated PMN showed decreased expression of activation markers. Moreover, ISA impaired degranulation and LPS triggered generation of ROS (6980 rfu ± 1338 vs 2870 ± 6989, *). FLU and VOR did not show a significant influence on PMN effector functions in vitro. MIC pretreatment resulted in enhanced expression of activation marker CD62L but reduced expression of CD11b, and decreased degranulation. Additionally, phagocytosis (27% ± 4 vs 44 ± 1, LPS, *) as well as generation of ROS (22660 rfu ± 3286 vs 41190 ± 2584, zymosan, *), and IL-8 synthesis were substantially impaired. CAS showed an increased phagocytosis (270% ± 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 alter any effector function. In vitro, treatment with POS 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: Fluconazole 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.

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CHARACTERISTICS AND OUTCOME OF PULMONARY INFILTRATES IN ACUTE LEUKEMIA CLASSIFIED ACCORDING TO EORTC/MSG CRITERIA OF INVASIVE FUNGAL INFECTION: A PROSPECTIVE STUDY BY THE RIL Study Group

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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 revealed to significantly affect the outcome of bloodstream infections (BSI) in AL patients (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 consecutive febrile/infectious episodes developing over a 26 months period in pts admitted to 9 hematological institutions within the Rete Ematologica Lombarda (REL) network.

Methods: From Dec-12 to Jan-14, all febrile/infectious episodes were recorded and data concerning PI extracted. PI were classified as specific and aspecific for IFI according to radiologic criteria.

Results: During 1069 episodes, 256 PI were diagnosed in 195 AL pts (M/F 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 (poss) 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. Prob/prov PI 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 215 (80.3% vs 92.9%, p=0.0164, and 56.1% vs 80%, p=0.0005, respectively), and higher frequency in patients on Fluorquinolone (Fq) prophylaxis (57.3% vs 22.9%, p<0.0001).

Multivariate analysis confirmed that aspecific IFI were less frequent during prolonged neutropenia (HR 0.392, IC 0.189-0.772), and poss IFI during Fq prophylaxis (0.344, 0.159-0.742). All but two patients (15.9% and 10%) had significantly higher in prob/prov IFI (31.3%, p=0.0192).

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 PI, 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 PI, 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.

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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 Aspergillus spp., Pneumocystis spp., and antifungal prophylaxis is an important consideration. Agents currently used for prophylaxis, voriconazole and TMP/SMX, carry safety and tolerability concerns. CD101 is a novel echinocandin in phase 2 clinical development that has demonstrated preclinical efficacy in treatment of invasive fungal infections and has physical and 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.

Materials and Method: C57Bl/6 mice (20 days old, 22-25 gm) were rendered neutropenic by cyclophosphamide (cypm) on day -4 (150mg/kg) and day -1 (100mg/kg) and challenged (day 0) with Candida albicans ATCC SCS314 (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%). 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/prov IFI (3.277, 1.243-8.644) predictive for death, as well as relapsed/refractory AL (2.45,1.092-5.498) and BSI (2.383, 1.092-5.498) predictive for death, as well as relapsed/refractory AL (2.45,1.092-5.498) and BSI (2.383, 1.092-5.498)
challenge on day -5, -3 or -1. Survival was monitored for 14 days. PCP model: C3HHeN mice (10 g) were immunosuppressed by dexamethasone (40 mg/kg) in acidified drinking water and inoculated with Pneumocystis murina (intranasal- ly, 2 x 10^4/50 μL). CD101 0.2, 2, or 20 mg/kg intraperitoneally was given at the time of inoculation and 1x 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 smallest group. All animals given higher doses survived regardless of day of prophylaxis. PCP: Trophic nuclei 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 in the outpatient setting and aspergillosis models suggests potential utility in the outpatient setting for treatment or prophylaxis.

P646 SURGICAL MANAGEMENT OF INVASIVE FUNGAL INFECTIONS IN ADULT LEUKAEMIA PATIENTS—EXPERIENCE FROM A LARGE TERTIARY CENTRE IN SOUTH-EAST ASIA

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Background: Invasive fungal infections (IFI) are a major cause of morbidity and mortality in patients undergoing chemotherapy or stem cell transplantation for acute leukaemias. Though optimized 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.

P647 INFECTIONS IN MULTIPLE MYELOMA ARE FREQUENT AND PREDOMINANTLY CAUSED BY BACTERIA: RESULTS OF A 12-YEAR SURVEY FROM A SINGLE CENTER

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Background: The outcome of patients with multiple myeloma (MM) has improved dramatically in the past years, mainly due to a better control of the disease. However, it is not clear what influence this has on treatment- or disease-related complications like infections. Recent data even suggested an increased rate of infections in patients with MM, possibly associated with the use of novel drugs.

Aims: To determine the rate and the type of infections in MM patients undergoing treatment and to evaluate possible disease- or treatment-related risk-factors.

Methods: All patients with MM treated at our institution between 2003 and 2014 were included in this retrospective analysis after approval by the institutional review board. Data on age, sex, diagnosis, comorbidities, treatment modalities, and infectious complications were recorded. Each type of therapy (e.g. high-dose therapy versus conventional therapy) defined a patient-case (duration per patient-case: beginning of therapy until the beginning of another type of therapy) and infections were recorded per case. To determine risk-factors, generalized estimating equations comparing cases were used.

Results: Four-hundred seventy-nine patients (male: 272, 57%) accounted for 1690 cases (median number of cases per patient 3, range 1-15). At presentation in our institution, median age was 62 (35-89) years, and most patients had advanced disease (Stage III according to Salmon-Durie classification in 364 patients, 76%). Median doses and an IqG paraprotein (255 patients, 53%). Type of therapy given were as follows: 534 (32%) conventional long-term chemotherapy, 514 (30%) induction-type chemotherapy, 237 (14%) chemotherapy for stem-cell mobilisation, 310 (18%) high-dose melphalan with stem-cell transplantation and 95 (6%) supportive care only. One-hundred sixty-six patients (35%) with 265 episode of infections never experienced an infection including 25 patients with high-dose melphalan. However, the majority of patients experienced at least one episode of infection throughout their treatment, accounting for 773 infections in 627 patient cases (37% of all patient cases). Most (559, 72%) infections were of bacterial origin including 156 cases with pneumonia (9% of all patient cases). Hemophagocytosis was noted in 105 cases of infections (11%, 95% Cl 1.5-2.5, p<0.001) and high-dose chemotherapy (OR 11.3, 95% Cl 8.4-15.3, p<0.001) were associated with a higher risk of infection whereas time of treatment (2003-2008 versus 2009-2014) or use of novel drugs did not influence the rate of infection.

Summary/Conclusions: More than 60% of MM patients experience at least one episode of infection during their course of treatment. These infections are mostly of bacterial origin and strongly associated with high-dose chemotherapy or relapse. Novel drugs do not seem to influence the rate of infection. Unfortu-
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 recognition molecules of the lectin pathway and especially ficolin-2 (L-ficolin) is emerging as an important component of the lectin pathway in the circulation. Ficolins share structural and functional characteristics with C1q from the classical pathway of the complement that acts with Pentraxin 3 (PTX3) that helps the innate immune system targeting pathogens like bacteria or viruses. In the context of hematopoietic stem cell transplantation polymorphisms of PTX3 have been identified as an individual risk factor for developing pulmonary aspergillosis.

**Aims:** We sought to investigate the impact of L-ficolin and PTX3 SNPs on the occurrence of infectious events such as sepsis and pneumonia, including invasive fungal disease (IFD), in 186 adult patients with newly diagnosed AML following anthracycline-based induction chemotherapy. In addition to our studies on membrane receptors, this work represents an important extension on soluble molecules of the innate immune system and their potential implication on infections.

**Methods:** Genotyping of L-ficolin and PTX3 SNPs (rs17514136, rs17549193, rs158250619, rs18540680) was performed by TaqMan assay. Multiple logistic regression analyses were applied to evaluate the association between SNPs of the polymorphisms and the occurrence of infectious events.

**Results:** Two L-ficolins SNPs were identified as risk factors for developing sepsis and/or pneumonia. Patients harboring rs17514136G/GAG or GG (n=100 or 22) revealed a significantly higher risk for developing sepsis (odds ratio [OR]: 1.88; 95% confidence interval [CI]: 1.01–3.37, p=0.039) or pneumonia (OR: 2.79; 95% CI: 1.1–6.9, p=0.033). A similar risk profile could be demonstrated for patients carrying rs17549193TT or TT (p=0.003). 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 and soluble molecules of the innate immune system might be helpful in identifying patients prone for infectious events.

**P649**

**PREDICTIVE FACTORS OF RESPONSE TO EPOETIN THETA IN CHEMOTHERAPY-INDUCED ANEMIA: A FRENCH MULTICENTER OBSERVATIONAL STUDY (PIVOINE)**


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**Background:** Whereas erythropoiesis stimulating agents (ESA) are indicated in the management of chemotherapy-induced anemia (CIA), their use in clinical practice is a matter of controversy with regards to some meta-analyses, opinions of national and international guidelines. However, supportive care is an area of high importance in onco-hematology and the absence of blood transfusion are independent predictive factors for complete response (OR 0.4 IC95% [0.335;0.478]). Moreover, good performance status (ECOG ≤ 1), history of transfusion, hematologic malignancy (excluding AML) and the absence of blood transfusion are independent predictive factors for complete response (OR 0.1.577 IC95% [1.186;2.098], OR 1.946 IC95% [1.459;2.597], OR 1.969 IC95% [1.411;2.747] respectively). Overall, only 27 patients (2%) experienced treatment-related adverse events, 2 of them (0.1%) presenting with a serious one (non fatal pulmonary embolism).

**Summary/Conclusions:** The PIVOINE study confirms that the response rate to epoetin theta varies considerably among patients treated similarly. This observational study conducted on a large population could help targeting the patients that could positively benefit from such treatment to prevent CIA, mainly patients with hematological malignancy, with good performance status and with low initial Hb level. The safety results confirmed the safety profile of epoetin theta.

**Results:** From November 2014 to October 2015, 1379 evaluable patients were followed in the study (mean age 68.3 ± 11.3 years, 47.2% men). Overall, 21.8% of patients presented with hematological malignancies, 19.9% with digestive tumors, 18.2% with lung cancer and 40.1% with other solid tumors. The majority had a good performance status (75.2% ECOG 0-1). More than 90% of patients had never received ESA prior to enrolment in this study and 45.2% benefited from pharmacological hemotherapy initiated at a level of ≥15, by Cochrane-Armitage test for trend across days. Causes of treatment delay were not assessed.

**Methods:** In an expanded-access protocol for patients with VOD/SOS post-hematopoietic stem cell transplantation (HSPCT): however, VOD/SOS can occur after chemotherapy induced HCT (VOD/SOS diagnosis to start of defibrotide for 1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher’s exact test and (2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8–14, and ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.

**Background:** Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSPCT); however, VOD/SOS can occur after chemotherapy induced HCT (VOD/SOS diagnosis to start of defibrotide for 1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher’s exact test and (2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8–14, and ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.

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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

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. Bronchoalveolar lavage fluid (BALF) was analyzed for cell count (bead-based by flow cytometry or by an animal blood counter), ELISA was performed for albumin amount and cytokines were analyzed by a multiplex assay. Bone marrow-derived PMN were isolated by magnetic cell sorting using biotin labeled Ly6G/C specific antibody. PMN functions were analyzed for degranulation, oxidative burst activity and CD62L shedding by flow cytometry. Fungal killing of PMN in vitro was assessed by a XTT assay. Chemotactic properties of A.f.-activated and control serum from wildtype and knock-out mice was evaluated by migration of purified human PMN, isolated by dextran sedimentation and Histopaque® centrifugation, in a transwell assay.

Results: While infected neutropenic mice developed lethal IPA, all wildtype mice survived the infection. Interestingly, Adams13+− mice displayed more severe signs of disease with a lethal course in about 24% of the animals. Examination of the lungs revealed a higher fungal burden along with increased signs of acute lung injury and levels of pro-inflammatory cytokines in ADAMTS-13 deficient. Histology sections demonstrated a more pronounced perivascular leukocyte infiltration in support of a dysregulated inflammatory response in Adams13+− mice. Importantly, we observed no general defect in the activation of neutrophil effector functions in response to conidia or hyphae in vitro. Furthermore, innate inflammatory response to IPA was not altered in VWF deficient (Vwf−/−) mice compared to wildtype (B6) control.

Summary/Conclusions: Therefore, we conclude that the proteolytic regulation of VWF by ADAMTS-13 or ADAMTS-13 by itself is an important mechanism to control PMN recruitment in acute inflammatory processes, such as fungal pneumonias.
Myelodysplastic syndromes - Biology

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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.

Results: We 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.

Methods: Using flow cytometry immunophenotyping, we quantitatively analyzed the different cell subpopulations within the CD34+CD38- and CD34+CD38+ HSPC compartments in 122 sequential MDS bone marrow samples obtained from 93 patients at different stages of HMA treatment.

Results: In line with earlier reports suggesting the presence of alterations in myeloid progenitor frequencies in MDS, our flow cytometry data stratified untreated patient samples in two groups representative of two abnormal differentiation patterns, which were independent of the IPSS risk classification. The CMP pattern group (12 samples, 34%) was characterized by an increased frequency of the common myeloid progenitors (CMP) (2.6-fold; p<10-4), whereas the LMPP frequency sharply increased (8-fold; p=10-4) in GMP pattern patients. The fact that a proliferative switch occurred in different HSC subpopulations confirmed that the two subgroups are distinct entities with different hierarchical origins.

Summary/Conclusions: Overall, our data provide evidence of the existence of biologically different MDS subtypes which are caused by separate differentiation defects and progress through the expansion of characteristic HSC populations.

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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-expressed 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 (Y891K) 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.

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 cooperation 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 expression 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-expressed 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-MT mutated myeloid malignancies.

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A NOVEL MASS SPECTROMETRY METHOD REVEALS THE INTRACELLULAR PHARMACOKINETICS OF AZACYTIDINE THERAPY IN VIVO
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Background: The cytidine analog 5’-Azacitidine (AZA, Fig A), a DNA demethylating agent, is the primary drug for the treatment of high-risk Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukaemia (CMML), and response is associated with improved survival benefits. However, only ~50% of treated patients will ever respond to AZA and the molecular basis for poor response is poorly understood. It is unclear whether non-responders to therapy have different rates of AZA uptake into their cells and/or AZA incorporation into nucleic acids compared to AZA responders, nor whether these might relate to DNA methylation in vivo. Aims: We aimed to develop an analytical method capable of simultaneously detecting all the subcellular fractions of AZA (Fig B) within the bone marrows of patients undergoing AZA therapy, while also assessing DNA and RNA methylation levels. This would provide the most comprehensive snapshot of the intracellular pharmacokinetics of AZA therapy in vivo as a first step towards better understanding AZA resistance.

Methods: We have developed a new method utilising mass spectrometry to accurately quantify all the different subcellular fractions of AZA within the same sample (Fig C). Using an Orbitrap mass spectrometer with very high mass resolution, we have achieved the first mass separation of DAC and AZA from all naturally occurring isotopes of 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 methylation. However, no such correlation was observed between AZA incorporation and RNA demethylation (Fig D). The sensitivity and resolution of our method also enabled, for the first time, a comprehensive survey of the total intracellular pharmacokinetics of AZA in vivo in patients undergoing a standard cycle of treatment. We discovered that the bone marrow cells of AZA responders (n=3) showed markedly higher levels of non-responders (n=2). DAC incorporation was also inversely proportional to DNA methylation levels, with lower DNA methylation 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 other non-responders (n=2) showed low or no DAC incorporation and no DNA demethylation (Fig E). Our method also enabled us to directly prove that low DAC incorporation was not a result insufficient AZA accumulation in cells, as cytoplasmic measurements of unincorporated AZA and DAC were higher 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.

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.

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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: We 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 RCMRD and 7 CMMML. Initially, WES was carried out on 40 diagnosis/progression-matched samples. Driver mutations were identified, after variant calling by a standardized bioinformatics pipeline, 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. Ten genes were recurrently mutated genes were SRSF2 (21%), TET2 (41%), STAG2 (28%), SF3B1 (21%), ASXL1 (21%), TP53 (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 during disease progression. 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 of type-1 mutations were detected at 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 (8/12), 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.

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PROGRESSION OF MDS TO AML FEATURES GAIN OF SINGLE DRIVER MUTATIONS WITH CONSEQUENT CHANGES IN CLONAL COMPOSITION AND OCCURRENCE OF MULTIPLE CLONES WITH MUTATIONS IN IDENTICAL GENES

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1Madrid, Spain, June 22 – 25, 2017
PRECLINICAL MODELING OF MYELODYSPLASTIC SYNDROMES

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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 RCMC, 4 RAEB-I, 2 RAEB II and 10 s-AML). In addition we analyzed 86 samples from healthy subjects stratified according to age (20-103 years) and 4 samples from beta thalassemia with iron overload. In all these samples, we evaluated the ATP/AMP ratio, as marker of energy status, the OXPHOS activity, in term of oxygen consumption and ATP synthesis, the lactate dehydrogenase (LDH) activity, as marker of anaerobic glycolysis, 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 data showed promising results with the injection of mononuclear cells obtained from patient BM, however the co-injection of mesenchymal stromal cells (MSCs) did not improve the level of engraftment. To address the question of the becoming of MSCs once injected, we tracked them back into the mice BM and showed that they disappeared after a week of engraftment. With a 2D in vitro system, we showed that we could co-culture CD34+ cells with the patient BM, on coverslips and non-adherent MSCs, over 4 weeks with a fold expansion ranging from 50 to 600 times. More importantly those cells conserved their clonal architecture and chromosomal aberrations.

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 MS5) as an alternative model to study MDS. This ex vivo culture system, which lasts for only 4 weeks and requires low number of human CD34+ cells, provides a robust preclinical assessment model to test therapeutic effects of different drugs and other approaches on the MDS clonality and autologous MSCs prior to treatment of MDS patients.

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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 FISM 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 RCMC, 4 RAEB-I, 2 RAEB II and 10 s-AML). In addition we analyzed 86 samples from healthy subjects stratified according to age (20-103 years) and 4 samples from beta thalassemia with iron overload. In all these samples, we evaluated the ATP/AMP ratio, as marker of energy status, the OXPHOS activity, in term of oxygen consumption and ATP synthesis, the lactate dehydrogenase (LDH) activity, as marker of anaerobic glycolysis, 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 data showed promising results with the injection of mononuclear cells obtained from patient BM, however the co-injection of mesenchymal stromal cells (MSCs) did not improve the level of engraftment. To address the question of the becoming of MSCs once injected, we tracked them back into the mice BM and showed that they disappeared after a week of engraftment. With a 2D in vitro system, we showed that we could co-culture CD34+ cells with the patient BM, on coverslips and non-adherent MSCs, over 4 weeks with a fold expansion ranging from 50 to 600 times. More importantly those cells conserved their clonal architecture and chromosomal aberrations.

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 MS5) as an alternative model to study MDS. This ex vivo culture system, which lasts for only 4 weeks and requires low number of human CD34+ cells, provides a robust preclinical assessment model to test therapeutic effects of different drugs and other approaches on the MDS clonality and autologous MSCs prior to treatment of MDS patients.

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PRECLINICAL MODELING OF MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders with diverse phenotypes, characterized by ineffective hematopoiesis and bone marrow morphological dysplasia with varying risk of leukemic transformation. Over the last decade, there has been significant progress in understanding the pathogenesis underlying the MDS. Notably, patient derived xenograft (PDX) models offer the most advanced clinical opportunity to capture the complexities of this myeloid malignancy. A number of studies have failed to achieve these goals and the more recent attempts to date are the NSG and the NSG-S (humanized with SCF, GM-CSF and IL-3).

Aims: Here we have used bone marrow cells from 39 MDS patients, covering all risk groups, to generate a preclinical in vivo and in vitro model, which could be used to study clonal evolution and test targeted therapies.

Methods: We have used NSG and NSG-S/GM3 mice to assess the scid-repopulating capacity of the MDS stem cells in presence or absence of mesenchymal stromal cells (MSCs). Moreover we have developed an in vitro 2D co-culture system as an alternative/complementary tool to in vivo studies.

Results: Our data showed promising results with the injection of mononuclear cells obtained from patient BM, however the co-injection of mesenchymal stromal cells (MSCs) did not improve the level of engraftment. To address the question of the becoming of MSCs once injected, we tracked them back into the mice BM and showed that they disappeared after a week of engraftment. With a 2D in vitro system, we showed that we could co-culture CD34+ cells with the patient BM, on coverslips and non-adherent MSCs, over 4 weeks with a fold expansion ranging from 50 to 600 times. More importantly those cells conserved their clonal architecture and chromosomal aberrations.
the controls (88 µM), suggesting an attempt to compensate the energy unbalance with the increment of anaerobic glycolysis. MDA level, which reflects the lipid peroxidation, is 1mM in young subjects, 9mM in elderly subjects, 9mM in b-thalassemia and 15mM in MDS. In vitro iron chelation partially restored 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 1.0. A similar result is observed in the LDH activity, 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 DFX treatment compared 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 normal controls.

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.

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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 (leukomalacia inhibitor [POM]), and MDS and AML patients. We further chose 13 of the differentially expressed genes for validation and characterization through the myeloid spectrum based on clinical correlation. i) druggable genes found to be upregulated in MDS cases, and therefore in our MDS patients or allowing for the inhibition of an specific DNA repair pathway (i.e. XPA, XRCC4, MSH4): ii) oncosenes infra-exposed 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 MDS 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 MDS patients compared with donors. Defects on genes predominantly unique to a single strand breaks repair pathway were identified.

Summary/Conclusions: Using an unbiased and massive DNA repair transcriptome assessment, we have identified a series of candidate targets for a synthetic lethality approach in CML. In addition, the different sense of mis-regulation of these and other targets within the myeloid diseases, some of them already being targeted in the clinical trial setting, improve the effect of the neoplasm-personalized test of DNA repair modulators.

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TRANSCRIPTOME ASSESSMENT OF DNA REPAIR GENES IN CHRONIC MYELOMONOCYTIC LEUKEMIA: SYNTHETIC LETHALITY TARGETS

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Background: Though genetic instability is a hallmark of myeloid disorders, the lack of recurrent somatic mutations, inarguably pathogenic, in the DNA repair machinery have precluded a predominant interest in this pathway. However, the recent discovery of non classical leukaemogenesis by splicing defects, the repair pathway influence on TET1 and TET2, and the development of unbiased high-throughput sequencing approaches oblige us to revisit those routes in blood cancers.

Aims: To perform improved massive RNA-seq in chronic myelomonocytic leukemia (CML) samples to identify neoplasm-specific targets for a synthetic lethality therapeutic approach. To validate the candidates through a direct strategy in an extended cohort of CMLM, myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) patients.

Methods: We performed enhanced RNA-seq on 27 CML bone marrow samples (13 del/del and 14 del/del) from 22 MDS patients and 90 healthy donors bone marrow from the MILE study.

Results: We validated these candidates through a direct strategy in an extended cohort of 73 additional CMLM patients and assessed their potential singular pattern in this disease by analyzing 80 MDS and 90 AML patients. We further chose 13 of the differentially expressed genes for validation and characterization through the myeloid spectrum based on clinical correlation. i) druggable genes found to be upregulated in MDS cases, and therefore in our MDS patients or allowing for the inhibition of an specific DNA repair pathway (i.e. XPA, XRCC4, MSH4): ii) oncosenes infra-exposed in our cohort but with inhibitory molecules already being tested in myeloid neoplasms.

Summary/Conclusions: Using an unbiased and massive DNA repair transcriptome assessment, we have identified a series of candidate targets for a synthetic lethality approach in CML. In addition, the different sense of mis-regulation of these and other targets within the myeloid diseases, some of them already being targeted in the clinical trial setting, improve the effect of the neoplasm-personalized test of DNA repair modulators.

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DIFFERENTIAL DIAGNOSIS BETWEEN MYELODYSPLASTIC SYNDROMES AND NON-CLONAL CYTOPENIAS BY FLOW CYTOMETRY ANALYSIS USING A MYELOID MATURATION DATABASE

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Background: The diagnosis of myelodysplastic syndromes (MDS) is based
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 standardisation 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 cytopneas using a myeloid maturation database.

**Methods:** Bone marrow samples from 55 MDS patients, and 51 controls with cytopenias using a myeloid maturation database.

**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 (90%) in the diagnosis of MDS (specificity 90%), but we also consider patients with scores above 3.5, thus achieving higher sensitivity (50%). Additionally, the number of immunophenotyping changes correlated well with prognostic risk. We confirmed that the higher the risk, 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 1).

**Figure 1.**

**Summary/Conclusions:** The maturation database (using the maturation analysis from Infinicyt® software) was useful to discriminate between MDS patients and non-clonal cytopenias, proving to be a reliable diagnostic test, also with prognostic implications. The application of this database as a diagnostic tool has the advantage that the result is independent of the observer. Inclusion of more myeloid markers and incorporation of erythroid parameters could increase sensibility in differential diagnosis.
LYMPHOPENIA IS AN INDEPENDENT RISK-FACTOR IN PATIENTS WITH P664 presentation.

Between epigenetic and immune therapy and the role of ERV-derived antigen

Little is known about their genome-wide transcriptional and epigenomic consequences.

Aims: To investigate the effects of epigenetic treatment on transcription and chromatin, we profiled genome wide transcription start sites (TSS) activities and epigenetically restored changes following the treatment with inhibitors against DNMTs, HDACs, or both.

Methods: Genome wide analysis of transcription start sites (TSS) (Cap analysis of gene expression (CAGE) sequencing), methylation status (whole-genome bisulfite sequencing) and chromatin dynamics (Chromatin-immunoprecipitation (ChIP) sequencing) was done by using a previously described reporter cell line model. Functional assays were used to investigate the mechanisms of LTR reactivation, a neuroblastoma mouse xenograft model to confirm the LTR reactivation in vivo.

Results: Following the treatment with inhibitors against DNMTs, HDACs, or both, we observed the activation of thousands of cryptic, currently non-annotated transcripts (TINATS). These TINATS arose most commonly from LTR12 elements, particularly LTR12C (ca. 50% of all TINATS). The resulting transcripts frequently splice into protein-coding exons and encode truncated or chimeric open reading frames which translated into currently uncharacterized protein isoforms with predicted abnormal functions or immunogenic potential, the last one based on their foreign sequence and capability of being presented on MHC-class I molecules. TINAT transcription after DNMTi coincided with DNA hypomethylation and HDACi with elevated histone acetylation marks, while HDACi specifically induced a subset of TINATS in association with H2AK9ac, H3K14ac, and H3K23ac. Despite this mechanistic difference, both inhibitors convergently induced transcription from identical sites since TINATS are encoded in solitary long-terminal repeats of the endogenous retrovirus family and are epigenetically repressed virtually all normal cells. Moreover, we found a consensus GATA2 binding motif which strongly distinguished LTR12C induced TINATS from from LTR12C without TINATS, supporting that GATA2 is likely the upstream transcription factor responsible for TINAT activation. Knock-down of GATA2 resulted in a reduced LTR12C expression despite epigenetic drug treatment. Overexpression of LTRs in our cell line model showed reduced cell viability in 3 out of 10 TINAT candidates. The reactivation of LTR12C elements upon epigenetic drug treatment could be confirmed in other malignant cell lines as well. Importantly, treatment with several chemotherapeutic agents did not affect LTR12C transcript levels, suggesting that their induction is a specific effect of epigenetic modulation rather than a general consequence of cellular stress. Additionally, we measured the transcription of LTR12C transcripts after SAHA treatment in a neuroblastoma mouse xenograft model, thereby confirming LTR12C induction in vivo.

Summary/Conclusions: DNMTi and/or HDACi induce de novo transcription of LTRs (LTR12 family), resulting in numerous fusion transcripts that encode novel protein isoforms which partly have the potential to influence cell proliferation, might explain the priming effect of epigenetic therapy and will be further investigated regarding their role as potential marker for epigenetic treatment response. Other future experiments will include proteomic approaches combining cell cytotoxicity assays to further shed light on the interaction between epigenetic and immune therapy and the role of ERV-derived antigen presentation.

IMPACT OF MARROW COMPLETE RESPONSE IN THE NATURAL HISTORY OF PATIENTS WITH MYELODYSTROPHIC SYNDROMES (MDS) AND CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) TREATED WITH HYPMETHYLATING AGENTS A. Alfonso Pierola1,*, G. Montalban-Bravo1, K. Takahashi1, E.J. Jabbour1, T. Kadia1, CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) TREATED WITH IMPACT OF MARROW COMPLETE RESPONSE IN THE NATURAL HISTORY P665

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 using the Kaplan-Meier analysis. Multivariate Cox regression analyses were performed.

Results: 2035 patients (RA n=182, RCMID =978, RARS =170, MDSdel5q n=92, RAEB-2 n=613) with a median follow-up of 23 months (mo) were identified. Data were sufficient for IPSS-R calculation in 651 patients. The mean absolute lymphocyte count (ALC) in the whole population was 1402/uL (95% CI: 1368-1437, range 0.12-4972) with no significant differences between the IPSS-R groups (very low-risk [n=77] mean 1471/uL, low-risk [n=255] mean 1406/uL, intermediate-risk [n=154] mean 1424/uL, high-risk [n=98] mean 1419/uL, very-high-risk [n=69] mean 1255/uL, p=0.067). 688 patients (34%) were lymphopenic (median ALC =790/uL) with a shorter survival (median 19.7 mo) versus 26.4 mo (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=0.039). With an ALC above the first quartile of the whole population (850/uL) as discriminator, the survival difference between lymphopenic and non-lymphopenic patients within the IPSS-R low-risk group reached statistical significance (survival median 67.4 vs 43.0 months, Log Rank p=0.002). This was not the case in the other IPSS-R subgroups. In multivariable analyses, an ALC <850/uL was an independent prognostic value for the IPSS-R low risk group after inclusion into a Cox regression model together with age <70 and LDH < normal value (240 U/l) (p=0.039). Patients with an ALC <850/uL had significantly lower platelet counts (median 97 versus 150 G/L, p=0.001) and neutrophil counts (median 1478 versus 1971/uL, p <0.001) counts but similar haemoglobin levels (median 121 versus 114 g/L, p=0.012).

Summary/Conclusions: An absolute lymphocyte count < 850/uL is an indep-
start of treatment was 21 months (95% CI=19-24); CR: 25 months (95% CI=20-30); PR: 27 months (95% CI=20-30); SD: 17 months (95% CI=14-19) (p=0.006). We compared OS between mCR vs CR (p=0.193, HR 0.796 [95% CI=0.765-1.122]), mCR vs PR (p=0.572; HR =0.564 [95% CI=0.378-0.810]) 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=13-18); 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=0.439-7.812]) and SD (p=0.7743; HR 1.059 [95% CI=0.752-1.491], but PFS was increased in those patients who achieve CR when compared to mCR (p=0.013; HR 0.665 [95% CI=0.482-0.918]) (Fig 1B).

Summary/Conclusions: Although mCR and CR result in the same OS, PFS is increased in patients achieving CR when compared with mCR. These data indicate that mCR should be considered as a valid endpoint in clinical trials.

Luspatercept increases hemoglobin and reduces transfusion burden in patients with lower-risk myelodysplastic syndromes (MDS): long-term results from phase 2 pace-MDS study


Background: Management of anemia is a common therapeutic challenge in patients (pts) with MDS. Luspatercept (ACE-536), a fusion protein containing modified action receptor type IIB (mAR-TIIB), is being developed for treatment of anemia in lower-risk MDS. 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) levels (Surugani R, Nat Med, 2014; Attie K, Am J Hematol, 2014). Aims: This ongoing, phase 2, multi-center, open-label study followed by a long-term extension (ext) study evaluates the effects of luspatercept in pts with lower-risk MDS. Endpoints include long-term safety and tolerability, erythroid response (IWG HI-E), RBC transfusion independence (RBC-TI, ≥8 weeks), duration of Hb-E, pharmacodynamic and iron metabolism biomarkers, and pt-reported quality of life (QoL).

Methods: Inclusion criteria: MDS IPSS low or int-1, age ≥18 yrs, Hgb <10 g/dL (if <4U RBC/8 weeks), no prior HMA, and no current lenalidomide or erythropoiesis-stimulating agent (ESA). The dose-escalation phase of the study is completed. An expansion cohort of up to 56 patients was added to this phase of the study to evaluate response and tolerability in pts who did not qualify for the phase 3 MEDALIST trial (for regularly transfused ring-sideroblast positive [RS(+)] patients with EPO >200 U/L). These include pts with low transfusion burden (LTB, ≤4U RBC/8 weeks) and either 1) RS(+) (≤15% in bone marrow) with baseline EPO ≤200 U/L or 2) RS(-) and any EPO level. RS(-) pts were also treated with ≥0.75mg/kg RBC-TI every 3 weeks subcutaneously for up to 5 doses (titration up to 1.75mg/kg) in the base study (NCT01749510) and are then eligible for long-term treatment up to 5 additional years (NCT02268383).

Results: Data were available for 73 base and 42 ext study pts. 37 (51%) and 19 (45%) base and ext pts were LTB and ≥20 ext/pts were LTB and ≥20 ext. RBC-TI was used for high transfusion burden (HTB, ≥4U RBC/8 weeks). Median (range) age (yr) was 72 (27-90), 53% pts had prior ESA, 51% pts had baseline EPO <200 U/L. Median (range) Hgb (g/dL) for LT pts was 8.6 (4.4-10.1). Median (range) RBC transfusion burden (U/8 weeks) for HTB pts was 6 (4-18). 71% base and 86% pts were RS(+) and 84% and 75% pts were RS(-) with EPO <200 U/L and EPO 200-500 U/L. RBC-TI rates for pts treated with ≥0.75mg/kg in the base and ext studies, respectively, were 62% (18/29) and 83% (19/23) for RS(+) pts with EPO <200 U/L and 46% (5/11) and 87 (185/18) for RS(+) pts with EPO 200-500 U/L. Preliminary RS(-) response rates (IWG HI-E and RBC-TI) by subgroup will also be presented at the meeting. Luspatercept was well tolerated, with related grade 3/serious adverse events (in 3 pts) as of 23Nov2016 of blast cell count increase, myalgia, and worsening of general condition. The most common grade 3/related AEs (≥2 pts) were diarrhea, fatigue, headache, hypertension, arthralgia, bone pain, injection site erythema, myalgia, and peripheral edema.

Summary/Conclusions: Lower-risk MDS patients treated long-term with luspatercept demonstrated robust and sustained increases in Hgb and decreases in transfusion burden and a high rate of RBC-TI. A Phase 3 study of luspatercept in regularly-transfused RS(+) patients with lower-risk MDS according to IPSS-R is ongoing (MEDALIST study; NCT02631070).

RATE AND CAUSES OF 5-AZACYTIDINE DISCONTINUATION AND SUBSEQUENT THERAPEUTIC OPTIONS IN 418 MDS PATIENTS FROM THE ITALIAN MDS REGISTRY OF FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE (FISM)


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Background: 5-Azacytidine (AZA) is the current standard of care for patients with high-risk myelodysplastic syndrome (MDS) in Europe. AZA has shown a
survival advantage when compared with conventional therapies and has also shown activity in IPSS lower-risk patients. However, about 40% of patients do not respond and most patients lose response within 2 years. Treatment options for MDS patients failing hypomethylating agents therapy are scarce and overall survival (OS) is extremely short.

**Aims:** Objectives of this study were to describe in a cohort of real life MDS patients treated with AZA, the reasons causing treatment discontinuation, and to evaluate the clinical outcome after the end of AZA therapy.

**Methods:** Unselected patients recorded in the MDS Registry of Fondazione Italiana Sindromi Mielodisplastiche (FISM) and treated with AZA from January 2009 to June 2014 were considered for the analysis. All types of conventional treatment regimens and combinations of AZA were allowed. Clinical response, cause of discontinuation, salvage treatment and OS from discontinuation of AZA were the major end points.

**Results:** Between January 2009 to June 2014 1799 newly diagnosed MDS patients were enrolled in the Registry, and 418 received AZA; 269 as 1st line treatment (64%), and 149 as a line ≥2 (36%). Median age at diagnosis was 73 years (range 8-91); 260 patients (62%) were male. WHO diagnosis was RA or RARS (n=27, 6%), RCMD or with or without RS (n=62, 15%) AAMES-1 (n=126, 30%), ARCE-2 (n=189, 45%), other subtypes (n=15, 4%). At start of AZA therapy IPSS score was low in 13 (3.4%), int-1 in 97 (23.2%), int-2 in 163 (43.8%), high in 67 patients (16%), and not available in 57 patients (13.6%). Patients received a median of 7 courses of treatment (range 1-63). Seventy-three percent of the whole cohort (418 pts) were alive at 1 year from beginning of AZA therapy and median OS was 23 months. (25 for IPSS lower-risk MDS and 21 for IPSS higher risk MDS). OS after discontinuation of AZA was 8 months. Clinical responses according to IWG criteria were available in 344/418 patients (82%). Of these 155 (45%) patients achieved a complete hematological response, 77 (22%) a partial response, 86 (25%) had stable disease while 36 (10%) did not respond. Response was achieved after a median of 6 cycles. After a median follow up of 16 months (range 7-35) in 37 (9%) patients AZA therapy was still ongoing while in 381 (91%) the treatment has been discontinued. Interruption of treatment was due to loss of response in 59 (16%) patients; AML evolution in 154 (40%), death in 43 (11%), toxicity or poor compliance in 39 (10%), allogeneic transplant (HSCT) in 12 (3%), other reasons in 22 (6%), not reported in 52 patients (14%). Of the 381 patients who discontinued AZA, 15 (4%) were managed with intensive AML-like chemotherapy, 22 (6%), received an allogeneic HSCT, 27 (7%) low-dose chemotherapy (7%), 22 (6%) erythroid stimulating agents, 18 (5%) other treatments and 277 (72%) patients no further treatment or only supportive therapy.

**Summary/Conclusions:** Our data confirm that AZA therapy is effective for MDS patients, both with higher and lower IPSS risk disease. Response rate is consistent with what previously reported, with a median OS of 23 months. Interestingly, at 16 months, 81% of patients had discontinued treatment, either for progression or loss of response and on only 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).

**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 was assessed and filtered by Mutect. Coverage was assessed using the IGV software. 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.

**Table 1.**
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 DECENTRALIZED MYELOMA (DEC-MM) FOLLOWING ASCT


Background: The role of autologous stem cell transplantation (ASCT) as first line therapy for newly diagnosed (ND) patients with multiple myeloma (MM) remains to be defined. Deterioration in the disease PFS for no-ASCT patients who respond to induction therapy may safely be assigned to delayed ASCT. 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 achieving ≥VGPR to induction may safely be assigned to delayed ASCT. Aims: This single arm phase 2 clinical trial conducted at 13 UK sites aimed to determine the progression free survival (PFS) for patients who achieved ≥VGPR to induction therapy with no further treatment. Here we report the primary analysis of the patients 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; doxorubicin 9mg/m2 days 1-4, dexamethasone 40mg days 1, 4, 8, 11). Co-variables assessed at 100 days post PBSCH (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(1;14), del(17p13), t(1q21)].

Results: Between April 2011 and January 2014 153 patients were enrolled (median age 55, range 28-71 years), 139 (91%) received 4-6 cycles of PAD. The majority (88.2%) received SC bortezomib, 18 (11.8%) received at least 1 IV bortezomib. At baseline, markers for disease burden, such as the percentage of total lymphocytes (15.4 ± 7.8), CD19+ B-cells (1.6 ± 0.9), CD38+ T-cells (0.5 ± 0.3) in BM were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 ± 7.8) and CD19+ B-cells (1.6 ± 0.9) were all higher in the good response cohort at baseline.
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Background: Lytic lesions occur in the majority of patients with multiple myeloma (MM) and represent one of the criteria for starting therapy. In the past, whole body X-ray (WBX) represented the method of choice for detecting skeleton 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, vertebral 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=1419) or smoldering multiple myeloma (N=11) observed at the AOUP, 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 anthracyclines (40%) were administered.

Results: Overall, we compared 160 PET-CT, 233 WBX, 106 CT, and 85 MRI exams. At diagnosis, PET-CT allowed detecting skeletal involvement in 18% of cases negative by WBX, in 37% of those CT-negative, and in 10% of those MRI-negative. Sensitivity of PET-CT was superimposable to that of MRI (90%), and higher than that of WBX (60%) and CT (73%). Nevertheless, the specificity was lower for PET-CT and MRI (40%) in respect of CT (51%) and WBX (71%). Analogously to that observed at diagnosis, PET-CT during follow-up showed distinct advantages in terms of sensitivity compared to X-rays (83% vs 60%, respectively). In contrast, PET-CT sensitivity was comparable to that of CT and MRI. As at diagnosis, the specificity was higher for WBX (70%) than for CT, RM and PET-CT (40% for all of these). When PET-CT was correlated to the quality of response, it was significant only in the not transplanted cohort (PR rate in PET-negative cases=87% vs 23% in the PET-Positive cases; p=0.016).

Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS.

Summary/Conclusions: Our study showed that PET-CT and MRI would represent the techniques of choice in the assessment of bone involvement in MM patients in view of their high and comparable sensitivity. Moreover, PET-CT gives the possibility of a "whole body" analysis in exchange for higher "biologic" cost.

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\textbf{INITIAL PHASE 2 RESULTS OF IBRUTINIB COMBINED WITH BORTEZOMIB/DEXAMETHASONE IN PREVIOUSLY TREATED PATIENTS WITH SMOLDERING OR MULTIPLE MYELOMA} R. Hájek\textsuperscript{1,2}, L. Pour \textsuperscript{3}, I. Špička\textsuperscript{4}, M. Ozcak\textsuperscript{5}, V. Maisnar\textsuperscript{6}, M. Turgut\textsuperscript{7}, L. Kwei\textsuperscript{8}, Z. Salman\textsuperscript{8}, E. Bilotti\textsuperscript{8}, A. Oriol\textsuperscript{9}
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Background: Bruton’s tyrosine kinase (BTK) is overexpressed in, and has been implicated in the growth and survival of multiple myeloma (MM) cells, providing a rationale for evaluating BTK inhibitors in MM (Yang Cancer Res 2015; Tai Blood 2012). Yang 2015 demonstrated that BTK overexpression (OE) contributes to blunted responses in MM cells when treated with widely used MM drugs (ie, bortezomib [BTZ], etoposide and doxorubicin). Increased activity of the ABC transporter efflux pump and expression of the ABCB1 transporter (dex) demonstrated an ORR of 38% in relapsed/refractory MM patients (pts) with a median of 3 prior therapies and 88% refractory to their most recent therapy (Chari ASH 2015), warranting further investigation of ibrituximab with proteasome inhibitors.

Aims: To evaluate safety and efficacy of combination ibr+BTZ+dex in previously treated MM pts.

Methods: In this phase 2, open-label, multicenter, European study (PCYC-1139), eligible pts received 1-3 prior therapies and demonstrated disease progression on or following the most recent therapy. Prior BTZ use was permitted provided pts were sensitive (ie, no progression 60 days after having achieved minimal response or better). All pts provided informed consent. For cycles 1-8 (21-day cycles), pts received ibrituximab 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 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 hematologic AEs occurring in >15% (>=3 pts) were: anemia (50%), lymphopenia (30%), and asthenia, peripheral edema, hypocalcemia and hypokalemia (20% each). Most Grade >3 AEs occurring in >1 pt were: thrombocytopenia (25%), asthenia and pneumonia (15% each), and hypotension, normal 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.

Summary/Conclusions: The initial data indicate promising clinical potential for the combination of ibr+BTZ+dex. Treatment was generally well tolerated without any unexpected safety signals noted for the combination. The preliminary ORR of 47% after a minimum 2 treatment cycles is encouraging with further follow-up needed.

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\textbf{PROGNOSTIC SIGNIFICANCE OF CLONAL CIRCULATING PLASMA CELLS BY MULTI-PARAMETRIC FLOW CYTOMETRY IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION} S. Sidana\textsuperscript{1}, N. Tandon\textsuperscript{1}, A. Dispenzieri\textsuperscript{1}, M.A. Gertz\textsuperscript{1}, F.K. Buadi\textsuperscript{1}, M.Q. Lacy\textsuperscript{1}, D. Dingli\textsuperscript{1}, A.L. Fonder\textsuperscript{1}, S.R. Hayman\textsuperscript{1}, M.A. Hobbs\textsuperscript{1}, Y.L. Hwa\textsuperscript{1}, P. Kapoor\textsuperscript{1}, R.A. Kyle\textsuperscript{1}, N. Leung\textsuperscript{1}, R.S. Go\textsuperscript{1}, J.A. Lust\textsuperscript{1}, S.J. Russell\textsuperscript{1}, S.V. Rajkumar\textsuperscript{1}, K. Kusein\textsuperscript{1}, W.I. Gonsalves\textsuperscript{1}
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Background: Presence of circulating plasma cells (cPCs) prior to autologous stem cell transplant (ASCT) is an adverse prognostic factor in patients with light chain amyloidosis (AL). Prognostic value of cPCs prior to ASCT in patients with light chain amyloidosis (AL) is not known.

Aims: The aim of our study was to evaluate if the presence of cPCs by multi-parametric flow cytometry (MFC) prior to ASCT is prognostic in patients with AL.

Methods: We retrospectively analyzed 130 patients diagnosed from 2008–2015 with AL who had cPCs analyzed by MFC prior to ASCT, and categorized them as follows: a) Group 1: patients proceeding directly to ASCT without induction therapy and b) Group 2: patients who received induction therapy before ASCT.
Results: There were 78 patients in Group 1 and 52 patients in Group 2. Patients in Group 2 had higher baseline 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 and 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 reached 98 months, p=0.96; PFS 43 vs 52 months, p=0.74). In multivariate analysis, adjusting for Mayo Stage and induction chemotherapy, there was a trend towards worse OS in patients with detectable cPCs (p=0.06).

Table 1.

<table>
<thead>
<tr>
<th>Group 1 (n=31)</th>
<th>Group 2 (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>60 (40-78)</td>
</tr>
<tr>
<td>Median dFLC (mg/L)</td>
<td>11 (0-50)</td>
</tr>
<tr>
<td>Median BMPC (106/mm3)</td>
<td>20 (6-90)</td>
</tr>
<tr>
<td>Median Mayo stage</td>
<td>2 (1-4)</td>
</tr>
<tr>
<td>cPCs at diagnosis</td>
<td>8 (26%)</td>
</tr>
<tr>
<td>cPCs at ASCT</td>
<td>5 (16%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: cPCs are cleared after induction treatment in majority of AL patients. Patients who have detectable cPCs prior to proceeding to ASCT after induction have worse PFS and OS than patients without cPCs. On the other hand, presence of cPCs was 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.

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RENAI IMPAIRMENT IN MYELOMA - PATIENT CHARACTERISTICS, TREATMENT MODALITIES, STEM CELL TRANSPLANT & OUTCOMES FROM THE AUSTRALIAN AND NEW ZEALAND MYELOMA REGISTRY

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Background: Renal impairment (RI) is a poor prognostic factor in multiple myeloma (MM). Analysis of disease characteristics, therapy & outcomes can improve treatment & prognosis.

Aims: To assess (1) characteristics of patients with RI at diagnosis - severity of RI, age, risk factors, high risk features, stage, disease manifestations & performance status, and (2) treatment including induction therapy & autologous stem cell transplant (ASCT) and outcomes.

Methods: Data from newly diagnosed MM patients enrolled in the Australian and New Zealand Myeloma Registry from 1 Feb 2013 to 31 Dec 2016 were analysed.

Results: Of 867 patients, 775 had eGFR available at diagnosis: 34% (287/775) had eGFR <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 (26% vs 46%, p=0.02). A minority of RI (66%) had advanced stage (ISS III) (6% 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 EOCG 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.

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 could 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.

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VENETOCLAX AS TARGETED THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA


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Background: Venetoclax (VEN), an orally available selective small-molecule
BCL-2 inhibitor, induces cell death in multiple myeloma (MM) cells, particularly those with the t(11;14) translocation.

Aims: The objectives of the study are to evaluate safety, PK, recommended phase two dose, and preliminary efficacy of VEN monotherapy in relapsed/refractory (R/R) MM.

Methods: Patients (pts) with relapsed/refractory (R/R) MM received VEN monotherapy in this phase 1 study. Daily VEN was given at 300–1200mg in dose escalation cohorts and 1200mg in the safety expansion. Pts with disease progression (PD) on VEN monotherapy could receive VEN plus dexamethasone and remain on study.

Results: As of 19Aug2016, 66 pts were enrolled. Median age was 63 years (33–83) and 30 (46%) pts had t(11;14). Median number of prior therapies was 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 6.1 months (range: 0.1–15.3). Fifteen-five (23.8%) pts discontinued, with 41 due to PD. Common adverse events (AEs) were nausea (47%), diarrhea (36%), vomiting (21%) and grade 3/4 hematologic toxicities (thrombocytopenia (32%), neutropenia (27%), anemia (23%), leukopenia (23%)). Common serious AEs were pneumonia (8%), sepsis (5%), cough, hypotension, pain, and pyrexia (3% each). There were no events of TLS. Six deaths were reported due to PD, and 1 each due to lung disorder and brain hemorrhage following trauma. Overall response rate (ORR) for all pts on VEN monotherapy was 21% (14/66); 10 (15%) achieved very good partial response (VGPR) or better [2 stringent complete response (sCR)]. 3 CR, 5 VGPR. For all pts, median time to progression (TTP) was 4.8 months (range: 0.4–9.7 months). A clear difference in responses was seen among pts with t(11;14) vs without [ORR, 40% vs 6%; vVGPR, 27% vs 6%]. For pts with t(11;14), median TTP was 6.6 months [vs 1.9 months for pts without t(11;14) and median DoR was 9.7 months]. 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 refractory to 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.

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GENE EXPRESSION CLASSIFIER EMC92/SKY92 AND REVISED ISS ROBUSTLY IDENTIFY HIGH-RISK MULTIPLE MYELOMA IN ELDERLY PATIENTS OF THE HOVON-87/NMSG-18 STUDY

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Background: Multiple myeloma (MM) affects mostly elderly people with a median age of 69 years at diagnosis, with 35-40% of patients older than 75. Overall survival (OS) is variable; of patients aged 66-79, 9% survive less than 3 months and 23% survive longer than 10 years. Recently the revised ISS (rISS) has been proposed as a prognostic marker that incorporates ISS, FISH and LDH. Another marker, the SKY92 prognostic classifier, was developed in younger, transplant eligible multiple myeloma (MM) patients who were included in the HOVON-65/GMMG-HD4 trial. The SKY92 classifier was thoroughly validated in eight independent cohorts, at the time of its initial publication, and since.

Aims: Here, we validated the SKY92 gene expression classifier and rISS in elderly, non-transplant eligible patients included in the HOVON-87/NMSG-18 trial (Zweegman et al. Blood 2016;127(9):1109-1116).

Methods: In this trial, melphalan, prednisone, thalidomide (MPT) plus thalidomide maintenance was compared with melphalan, prednisone, lenalidomide (MPR) plus lenalidomide maintenance. The MPMprofiler™ CE IVD assay was used to obtain SKY92 scores, classifying a patient as high-risk or standard-risk. In addition, the international staging system, LSR, FISH and rISS were analyzed.

Results: The 178 patients in the analysis for which enough bone marrow was available to perform GEP, had a median age of 73 years. At the time of data analysis, median follow-up was 23 months. A total of 31 patients (18%) and 25 of 178 patients as high-risk (14%). The median OS for the 25 patients classified as SKY92 high-risk was shorter than the median OS of standard-risk patients: SKY92 high-risk 21 months versus SKY92 standard-risk 53 months (hazard ratio (HR)=3.0, 95% confidence interval (CI)=1.7-5.3; p<0.01; Figure 1). The proportion of patients classified as high-risk (18%) is comparable to the 10% identified in the initial report of the rISS. Interestingly, the proportion of SKY92 high-risk patients is larger (14%), whereas the median OS associated with these patients is shorter (21 vs 25 months). The SKY92 classifier performed better compared to the rISS as high-risk marker for OS. The 2-year OS rate using the SKY92 classifier (71%) was significantly higher than for the rISS (52%) (p=0.016). The 2-year progression free survival (PFS) rate was similar for SKY92 high-risk and rISS-III (16% and 17%, respectively). In the multivariate analysis, SKY92, rISS and deletion of 13q were independently associated with OS. Inde-
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: A total of 13,718 patients with multiple myeloma were included in the study and 21 groups of comorbidities were identified. The most common diseases were cancer, hypertension, heart failure, ischemic heart disease and atrial fibrillation. Among all patients, 55% had no prior history of comorbidity, 23% had one comorbidity, 12% had two comorbidities, and 10% had three or more comorbid conditions. Survival was negatively influenced by the number of comorbidities (Figure 1). The risk of death was significantly increased in patients with atrial fibrillation (HR=1.08; 95% CI 1.00-1.16), heart failure (HR=1.50; 95% CI 1.40-1.61), stroke (HR=1.20; 95% CI 1.11-1.30), psychological disease (HR=1.27; 95% CI 1.16-1.39), chronic lung disease (HR=1.22; 95% CI 1.12-1.32), diabetes (HR=1.14; 95% CI 1.04-1.36), peripheral vascular disease (HR=1.26; 95% CI 1.12-1.42), cancer (HR=1.10; 95% CI 1.04-1.16), dementia (HR=1.65; 95% CI 1.38-1.99), paralyses (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).

Figure 1.

**Table 1. Multivariate survival analysis in the HO87/NM18 trial.**

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>1.10 (1.04-1.16)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.14 (1.08-1.22)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1.20 (1.12-1.28)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>1.27 (1.16-1.39)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1.08 (1.00-1.16)</td>
<td>0.045</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.20 (1.11-1.30)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Psychological disease</td>
<td>1.27 (1.16-1.39)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>1.22 (1.12-1.32)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.14 (1.04-1.36)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>1.26 (1.12-1.42)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cancer</td>
<td>1.10 (1.04-1.16)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Dementia</td>
<td>1.65 (1.38-1.99)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Paralyses</td>
<td>1.44 (1.15-1.80)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>1.38 (1.08-1.74)</td>
<td>0.0001</td>
</tr>
<tr>
<td>End stage renal disease</td>
<td>1.57 (1.03-2.04)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1.64 (1.14-2.43)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** Here, we compared the SKY92 classifier with revised ISS staging and FISH. These data validate the SKY92 classifier as a robust marker to identify high-risk patients in non-transplant eligible MM patients. In these IMiD treated patients, the SKY92, the revised ISS, and FISH markers such as deletion of 13q retain independent prognostic value.

**P678**

**MULTIPLE MYELOMA AND COMORBIDITY: A POPULATION-BASED STUDY**

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**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 1990 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:** A total of 13,718 patients with multiple myeloma were included in the study and 21 groups of comorbidities were identified. The most common diseases were cancer, hypertension, heart failure, ischemic heart disease and atrial fibrillation. Among all patients, 55% had no prior history of comorbidity, 23% had one comorbidity, 12% had two comorbidities, and 10% had three or more comorbid conditions. Survival was negatively influenced by the number of comorbidities (Figure 1). The risk of death was significantly increased in patients with atrial fibrillation (HR=1.08; 95% CI 1.00-1.16), heart failure (HR=1.50; 95% CI 1.40-1.61), stroke (HR=1.20; 95% CI 1.11-1.30), psychological disease (HR=1.27; 95% CI 1.16-1.39), chronic lung disease (HR=1.22; 95% CI 1.12-1.32), diabetes (HR=1.14; 95% CI 1.04-1.36), peripheral vascular disease (HR=1.26; 95% CI 1.12-1.42), cancer (HR=1.10; 95% CI 1.04-1.16), dementia (HR=1.65; 95% CI 1.38-1.99), paralyses (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:** In this large, population-based study including almost 14,000 patients, we have shown that comorbidities are common among newly diagnosed multiple myeloma patients and that comorbidities are associated with an inferior survival. Importantly, the number of comorbidities showed a dose-response relationship with inferior overall survival. For example, the median overall survival for patients with 3 or more comorbidities was reduced by more than 50% compared to patients without comorbidities. The importance of comorbidities should be taken into account when evaluating patients and deciding on treatment strategies for individuals with multiple myeloma.
P679 DETECTION OF NEW EMERGING CLONES DURING TREATMENT BY NGS ALLOWS A BETTER RISK PREDICTION ON MULTIPLE MYELOMA PATIENTS

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Methods: Immunohistochemistry with 1-2% of positive staining for CD38 was analyzed in tissue sections of 137 patients (pts) with progressive disease (PD) treated with or without daratumumab. Other pts received lenalidomide (LEN) or high dose melphalan (HDM) as 1st line or lenalidomide/lenalidomide as 2nd line or 3rd line + dexamethasone (DEX). The pts were selected among those who had been treated at least twice with daratumumab and for whom the staining with CD38 was available. The data were collected using a database that included all the pts treated with daratumumab in different settings at the Genentech Suisse site. The database was reviewed for the CD38 expression and the clinical data were collected from medical records.

Results: The analysis included 137 patients with CD38 expression at baseline and at follow-up. At baseline, 55 (40%) pts showed CD38 expression ≥10% and 82 (60%) pts ≤10%. At follow-up, 112 (82%) pts showed CD38 ≥10% and 25 (18%) pts ≤10%. The median time to loss of expression was 8 months for patients with ≥10% at baseline. Of the 137 pts, 112 (82%) showed an increase in CD38 expression ≥10% vs ≤10%. In patients with ≥10% at baseline, the median time to progression (TTP) was 10 months for patients with CD38 ≥10% vs 3 months for patients with CD38 ≤10%. The difference was statistically significant (p=0.002).

Conclusion: CD38 expression may be a potential predictive biomarker for daratumumab and can be used to select patients and subgroups that may benefit from daratumumab treatment.
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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1AbbVie, Inc., North Chicago, 2Genentech Inc., San Francisco, United States

Background: The anti-apoptotic proteins BCL-2 and MCL-1 have been shown to promote resistance to numerous chemotherapeutics. 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), 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.

Figure 1.

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 prior lines of therapy compared to 4 or more lines of therapy (median: 3.03 vs 0.94, p<0.01). In contrast, no association was observed between BCL2L1 or MCL1 gene expression and response or number of prior therapies. Bootstrapping and aggregating thresholds from trees was used to estimate a threshold value for BCL2 expression that would provide optimum selection of patients who achieved a response. 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 3.0 months) were also longer for patients with high compared to low BCL2 expression. Responses in high BCL2 expressers 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.

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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 dependency, 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 switching of RRT or hospital, 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).

Figure 1.

Summary/Conclusions: 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.

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TREATMENT WITH POMALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH MULTIPLE MYELOMA AND LIGHT CHAIN (AL) AMYLOIDOSIS

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Background: Multiple myeloma (MM) is a progressive, incurable disease with a median survival of 10 years. Despite recent treatment progress, there is still a significant unmet need for highly effective treatments for patients failing to respond or achieving only short-term remission to front-line therapy. The incorporation of immunomodulatory drugs (IMiDs) into both autologous stem cell transplantation (ASCT) and maintenance regimens has demonstrated a significant impact on overall survival in MM patients. In the last years, patients with multiple myeloma (MM) and amyloidosis have been treated with the novel agent pomalidomide, a third generation of immunomodulatory drugs (IMiDs) which seems to be more effective than thalidomide and lenalidomide in terms of objective response rate.

Aims: The purpose of this study was to evaluate the efficacy and safety of pomalidomide and dexamethasone treatment in patients with MM and AL amyloidosis.

Methods: A total of 17 patients (9 males and 8 females, median age 69 years) were treated with pomalidomide and dexamethasone. The median number of previous lines was 4 (range 1–7). The treatment consisted of pomalidomide 2 mg on days 1-21 and dexamethasone 20 mg on days 1–3, 8–10.

Results: The median response duration was 12 months (range 3–24). All patients achieved a partial response or better. Overall survival was not reached at a median follow-up of 24 months (range 3–60). Two patients died during the treatment due to disease progression. A third patient died due to a non-disease related cause.

Summary/Conclusions: Pomalidomide and dexamethasone treatment is an effective and well tolerated regimen in patients with MM and AL amyloidosis. Further studies are needed to determine the optimal dosing regimen and to evaluate the long-term efficacy and safety of this combination.
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.

Aims: 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 Amyloidosis Research and Treatment Center were searched for patients with a diagnosis of multiple myeloma and AL amyloidosis (amyloid fibrillography and/or histology positive). Among them, 3 were diagnosed of AL amyloidosis, both by IHC and histology. Two remaining patients were diagnosed of AL amyloidosis by IHC unequivocally in other tissues. Mean value of NT-proBNP (N-terminal natriuretic peptide) in patients with AL amyloidosis was 7730 pg/mL for those with a positive uptake in the scintigraphy and 9990 pg/mL for those with a negative uptake.

Summary/Conclusions: Cardiac 99mTc-DPD SC has been described as a useful technique in the differential diagnosis between AL and TTR amyloidosis. However, up to 30% of cases of AL amyloidosis show some degree of uptake and 10% show a pattern consistent with TTR amyloidosis (biventricular uptake and PS 2-3). In our cases have been described with myocardial deposit of both TTR and light chain and only 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 the 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.

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MYOCARDIAL UPTAKE OF 99mTc-DPD IN PATIENTS WITH AL AMYLOIDOSIS

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Background: AL amyloidosis is a free 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 the detection of cardiac amyloid. However, it is not widely used and PS 2-3 (Perugini score 2-3 (PS)) is highly suggestive of TTR amyloidosis. An intense biventricular uptake (Perugini score 2-3 (PS)) is highly suggestive of TTR amyloidosis. The aims of this study were to evaluate the diagnostic value of 99mTc-DPD scintigraphy in the differential diagnosis between AL and TTR amyloidosis and to assess the impact of this technique on patient management.

Methods: The databases of the Pavia Amyloidosis Research and Treatment Center were searched for patients with a diagnosis of multiple myeloma and AL amyloidosis (amyloid fibrillography and/or histology positive). Among them, 3 were diagnosed of AL amyloidosis, both by IHC and histology. Two remaining patients were diagnosed of AL amyloidosis by IHC unequivocally in other tissues. Mean value of NT-proBNP (N-terminal natriuretic peptide) in patients with AL amyloidosis was 7730 pg/mL for those with a positive uptake in the scintigraphy and 9990 pg/mL for those with a negative uptake. 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 PS 2-3). In our cases have been described with myocardial deposit of both TTR and light chain and only 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 the 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 GEN501 (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 at least one 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 963 treatment lines from 550 patients. The relative 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 IMIDs and PI/IMiD refractory status. Clustering of observations at the treatment-line level within patients was controlled for using the robust sandwich estimate for the covariance matrix. Statistical significance testing was performed using a two-tailed P-value 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 characteristics included in the multivariate model between the DARA and SOC groups, results showed a significant improvement in favor of DARA compared with SOC for OS (HR=0.42 [95% CI 0.31–0.57]). When limiting the comparative analysis to European patients from the IMF cohort (n=341), results for OS are very similar (HR=0.40 [95% CI 0.28–0.58]).

Summary/Conclusions: Findings from the regression analyses using the updated IMF dataset were consistent with results from the previous 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:

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PREDICTORS OF EARLY DEATH RELATED TO ACTIVE MULTIPLE MYELOMA IN ELDERLY PATIENTS RECEIVING OPTIMIZED FRONTLINE TREATMENT COMBINATIONS


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Background: Multiple Myeloma (MM) is predominantly a disease of the elderly and the outcome of these patients is poorer than that of transplant candidates. It is well established that those considered frail or unfit have a dismal prognosis, however, even within fit patients, such as those included in clinical trials, there is substantial proportion of early deaths (within the first 2 years after diagnosis). Identification of this “high-risk” fit elderly patients could contribute both to the design of innovative clinical trials, and to avoid the emotional and economical burden of inactive and futile treatments.

Aims: To analyze the factors associated with early death (within first 2-years) due to active MM in elderly newly diagnosed (NDMM) patients fit enough to be included in clinical trials with optimized therapy with proteasome inhibitors and IMiDs.

Methods: 497 NDMM not transplant candidates treated in two prospective GEM-PETHEMA trials were included in the study; GEM05MAS65 (n=260) used frontline treatment with either bortezomib-melphalan-prednisone (VMP) or bortezomib-thalidomide-prednisone followed by maintenance with bortezomib, thalidomide or bortezomib, prednisone; the GEM2010 MAS68 (n=239) compared induction with sequential or alternating cycles of VMP + lenalidomide-dexamethasone. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Table 1.

Results: From the 497 patients included, 77 (15%) patients died within 2 years from diagnosis due to active MM. When we compared this latter cohort with the remaining patients, the profile of the high risk group was characterized (Table 1) by a higher proportion of patients >75 years, advanced ISS and R- ISS stage, higher β2-microglobulin (β2-M) levels (>3.5 and 5.5mg/dl) and abnormal LDH; increased incidence of high-risk cytogenetic features (HR CA), CD45- clonal plasma cells, and lower incidence of CD27+ MM phenotype. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

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 punctuation ≥5 segregates a subgroup of patients with poor outcome (PPV: 83.3%, the NPV: 84.02%).

Summary/Conclusions: The risk of early death due to active disease in elderly patients was related to four independent prognostic factors: age >75y, high LDH levels, advanced ISS, and presence of HR CA. A score ≥ 4 identify a subgroup of patients with high probability of death within 2 years despite optimized treatment.

Myeloproliferative neoplasms - Biology

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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. 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 2009; 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 MPLhigh subpopulation was clearly separated from the SLAMF7low MPLlow population in human CD14+ monocytes. A significantly higher frequency of fibrocyte differentiation was observed in the SLAMF7high MPLhigh population. The number of SLAMF7high MPLhigh cells was significantly greater in MF patients than in healthy donors. Conversely, their numbers did not increase in MF patients treated with ruxolitinib.

Summary/Conclusions: MPL activation directly induced fibrocyte differentiation from monocytes and macrophages expressing MPL, and the elimination of these cells reversed the MF phenotype. Our findings confirmed a link between fibrocytes and the TPO/MPL signaling pathway and indicated that the combination of MPL and SLAMF7 could be a useful fibrocyte marker in monocytes or macrophages.

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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-

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duced growth factors. However, in other tissues and organs, fibrosis is associated with monocyte/macrophage infiltration, 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 (FMF) (Verstovsek, J Exp Med. 2016). We demonstrated that the BM of FMF patients harbors more neoplastic, functionally distinct fibrocytes and fewer MS-FMCs compared to hematologically normal bone marrow. In addition, we detected an overabundance of fibrocytes in the BM and spleen of an established FMF mouse model and a xenograft mouse model of FMF created using BM-derived low-density cells from patients with FMF.

Aims: Fibrocytes, which make up <1% of BM cells, differentiate from a subpopulation of CD14+ monocytes and are recruitable 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 FMF.

Methods: To test this hypothesis, we transplanted NSG mice (NOD/Scid (NoD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ)) with sorted CD14+ monocytes from patients with JAK2V617F-positive PMF or donors with hematologically normal BM.

Results: Here, we show that BM-derived CD14+ cells from patients with JAK2V617F-positive PMF or donors with hematologically normal BM engrafted in NSG mice. Transplanted NSG mice with PMF BM-derived CD14+ monocytes developed a myelofibrosis-like phenotype with reticulin fibrosis and abundant neoplastic (JAK2V617F) fibrocytes in the BM and spleen. Two months after transplantation, we detected a subpopulation of 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. Importantly, we established an embedded BM section 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.

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ESTABLISHMENT OF AN IN VITRO MODEL FOR THE SKewed MEGAKARYOPOIESISty BY CALRETICULIN MUTATION IN HUMAN CELLS

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Background: Somatic mutations on calreticulin (CALR) gene are found in a majority of patients with JAK2-unmutated MPN, and are associated with increased alpha-growth factor receptor and 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 iPSC cells (iPSC) established from an essential thrombocythemia (ET) patient and a healthy individual harboring a 5-base insertion mutation in the CALR gene, as well as alpha-growth factor receptor-negative hematopoietic progenitor cells (HPCs) were produced from iPSC by “iPS-Sac” method. HPCs were then cultured to induce megakaryocytic cells (MKs) and erythroid cells defined by CD42b and CD235a, respectively. The mechanism of skewed differentiation was assessed by measuring the mRNA expression of lineage-determinant genes such as FLI1 and KLF1. To demonstrate that established assay system for the use of compound screening, CALR ins5-dependent megakaryopoiesis was examined by therapeutic compounds.

Results: The number of CD34+ HPCs produced from iPSC was unchanged between CALR ins5 and CALR wt genotypes, implying that CALR ins5 did not affect HPC differentiation from iPSC. However, upon exposure to activating activation of MPl by mutant CALR, MKs were induced from CALR ins5-HPC even in the absence of TPO in either semi-solid or liquid culture systems, which was not evident with CALR wt-HPC. Unlike megakaryopoiesis, both CALR ins5 and CALR wt HPCs required EPO for the production erythroid cells. However, the number of erythroid cells differentiated from CALR ins5-HPC was slightly decreased compared to that from CALR wt HPC, implying that CALR ins5 interfered with the erythroid cells differentiation from iPSC-derived HPC. This interference was seemingly caused by a premature expression of FLI1 that blocked KLF1 expression required for the erythroid 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.

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QUANTITATIVE PROTEOMIC 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 (ET and primary thrombocythemia variant ET(PT)), essential thrombocytosis (ET), and 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), known 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 JAK2 kinase activity and could be regulated by JAK2 kinase inhibitors.

Summary/Conclusions: In conclusion, these results reveal proteome alterations in MPN granulocytes depending on the phenotype and genotype of patients, highlighting new oncogenic mechanisms associated with JAK2 mutations and overexpression of calreticulin.

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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 hematologic 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 are resistant against several tyrosine kinase inhibitors (TKI), including imatinib. DCC-2618 is a novel switch control inhibitor that has been described to block the JAK2 kinase activity from its active site and inhibit autophagy.

Aims: The aims of this study were to evaluate the effects of the switch control inhibitor DCC-2618 on proliferation and survival of neoplastic MC and other hematopoietic cells in various conditions. The effect on hematopoietic cells from patients with SM was also assessed.

Methods: we used different human MC lines (HMC-1.1, HMC-1.2, ROSAKITWT, ROSAKITD816V, ROSAKITK509I, MCPV-1.1, MCPV-1.2, 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
MOLM-13, MV4-11, KG-1 and U-937, the eosinophilic leukemia cell line EOL-1, human cultured umbilical vein endothelial cells (HUVEC), the microvascular human endothelial cell line HMEC-1 and primary neoplastic cells obtained from patients with AML, chronic myelomonocytic leukemia (CMML) and (clonal or reactive) hypereosinophilia were used. Cell proliferation was quantified by ³H-thymidine uptake. Apoptosis was determined by flow cytometry and light microscopy. The phosphorylation status of Stat5 and BTK was analyzed by Western blotting. The effects of DCC-2618 on histamine secretion in basophils (BA) were analyzed by histamine release assay.

Results: DCC-2618 was found to block the proliferation of all MC lines tested, with lower IC₅₀ values measured in KIT D816V-negative HMC-1.1 cells (12±3.7 nM) and ROSA²²KITWT cells (4±1.5 nM) than in KIT D816V-positive HMC-1.2 cells (123±36 nM), ROSA²²KITD816V cells (183±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 SM and with ASTH. The mean age of patients with SM was 60 ± 8 years and the mean age of patients with ASTH was 62 ± 8 years. DCC-2618 induced apoptosis and blocked tyrosine phosphorylation of Kit in all MC lines tested. We were also able to show that DCC-2618 inhibits proliferation and survival in the eosinophilic leukemia cell line EOL-1 (IC₅₀ 1.8±1.3 nM) and the FLT3 ITD-mutated AML cell lines MV4-11 (IC₅₀ 147±80 nM) and MOLM-13 (IC₅₀ 132±56 nM). In addition, DCC-2618 was found to block proliferation in primary leukaemic cells in patients with monoblastic AML and CMML which are 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-ϕ IL-3-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).

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DISTRIBUTION OF MUTATIONS IN DRIVER AND NON-DRIVER GENES ACCORDING TO CLONAL HEMATOPOIESIS IN ESSENTIAL THROMBOCYTHESIS 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 age median of 60 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 6 patients (17 PV and 28 ET). The most frequently mutated genes were TET2 (16%), DNNM3A (8%), ASXL1 (5%), SF3B1 (5%), EZH2 (2%) and RUNXI (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 (Biallelic JAK2 n=1, JAK2 + CALR n=5, CALR + MPL n=3, and MPL + ASXL1 n=1). In 12 cases the VAF of the driver mutation (JAK2 n=9, CALR n=1, MPL n=2) was similar to the non-driver mutation, being TET2 the codominant mutation in 6 of them. HUMARA analysis was clonal in 66 patients and polyclonal in 60 patients (30%). JAK2V617F was most frequently co-expressed in ET (66% vs 14% in PV, p=0.002). Clonal HUMARA was observed in 90% of MPL-mutated patients in comparison with 58% in JAK2-mutated, 42% in CALR-mutated and 11% in TN (p=0.0001). Two patients with TN ET showing clonal hematopoiesis had TET2 mutations. In JAK2-mutated women, the mutant allele load was significantly higher in constitutional cases (43% vs 23%, p=0.02) and in PV than in ET (76% vs 47%, p=0.01). Eighty percent of patients with non-driver mutations showed HUMARA clonality vs 37% of patients without non-driver mutations (p<0.0001). The mutated genes significantly associated with a higher frequency of clonal hematopoiesis were TET2 (p=0.007) and SF3B1 (p=0.029).

Background: Myelofibrosis (MF) is the myeloproliferative neoplasm chromosome Ph-negative with worst prognosis. MF is characterized by stem cell-derived clonal myeloproliferative and reactive cytokine-driven inflammatory bone marrow fibrosis. Ruxolitinib is the first line treatment for MF. It was associated with significant reduction in symptomatic spleenomegaly and improved constitutional symptoms. In a previous work (Arenas et al. Blood Volume 122, Issue 21 (ASH Annual Meeting Abstract)) we identified a set of promising synergistic drugs combinations for a ruxolitinib. Nilotinib and prednisolone were selected from them.

Aims: The aim of this work is the study the effect of the combination of ruxolitinib, nilotinib and prednisolone in hematopoietic progenitor cells from patients with MF.

Methods: A ruxolitinib, nilotinib and prednisolone dose-response curves and synergistic studies were performed in hematopoietic progenitors CD34⁺ from five MF patients. We studied the molecular effect of single drugs and in combination on SET2 cell line with western blot. To adress the antiangiogenic activity of the drugs and their combinations, we pre-incubated HS27 cultures with 100nM of ruxolitinib, 1µM of nilotinib, 1µM of prednisolone or their combination during 1 h. After that, we added 2ng/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, 6.5µM and 13.1µM, respectively. A combination index (CI) of less than 1 indicated synergy. All combination had a synergistic behavior (Table 1); moreover, there were two combinations whose CI from all samples were less than 1: 32nM ruxolitinib plus 0.8µM prednisolone (CI=0.25±0.11) and 32µM nilotinib plus 0.8µM prednisolone (C=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 untreated cells. 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 untreated cells. 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 untreated cells.
% (p-value<0.05) by ruxolitinib, 42.6±14.4 % by RN and 70.8±11.2 % by RNP (p-value<0.001). This inhibition was maintained at 3 hours by ruxolitinib (57.5±25.2 %), nilotinib (38.4±26.8 %), RN (30.5±24.03 %) and RNP (37.4±16.5 %). Then, the antifibrotic activity of the drugs and their combinations were studied. Nilotinib reduced the mRNA expression of COL1 by 48.1±29.9 % (p<0.05) and prednisolone (RN: 37.8±1.9 % (p-value<0.05). These results were corroborated by ICC: the inhibition of expression of collagen I was more intense if the HS27 were treated with nilotinib or RN (figure 1).

Summary/Conclusions: In conclusion, ruxolitinib, nilotinib, prednisolone and their combinations had a synergistic behavior to control the proliferation of myeloid cells in MF patients; moreover, they had antifibrotic activity in fibroblast cells. For these reasons, the combined ruxolitinib/nilotinib/prednisolone could be a promising therapy to MF and support an ongoing clinical trial in MF patients.

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INTERLABORATORY ASSESSMENT OF MUTATION DETECTION IN MYELOID MALIGNANCIES BY TARGETED NEXT-GENERATION SEQUENCING

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Background: Next-generation sequencing (NGS) technology is being implemented in clinical practice for assessing the mutational status of myeloid neoplasms. The Working Group on Molecular Biology from the Spanish Society of Hematology has performed an interlaboratory assessment of gene mutation analysis by targeted NGS using myeloid panels.

Aims: To assess the technical performance of mutation detection by targeted NGS using myeloid panels.

Methods: The technical comparison was established on two rounds with samples previously analysed using NGS panels, Sanger sequencing and/or fragment analysis. First, four DNA samples (S1-S4) from AML patients were shared among laboratories. For the second round, five DNA samples (S5 to S9) were shared among 14 laboratories. The center of origin had previously characterized and confirmed: for the first round, 14 relevant mutations in 10 genes; and for the second round 17 relevant mutations in 7 genes. Each center performed laboratory preparation, sequencing and blind variant analysis following their own routine practice. Detected variants and data regarding main methodological parameters were collected. Detection rate was calculated as the number of laboratories with positive detection out of the number of laboratories that sequenced the particular gene region.

Results: Eight different gene panels were used for laboratory preparation (pre-determined in 10 labs and custom in 4). The predominant approach was amplicon enrichment (11/14, 78.6%) and only 3/14 laboratories (21.4%) used capture-based methods. Sequencing was performed with Illumina devices in 9/14 laboratories and Ion Torrent platforms in 5/14. Alignment and variant calling was performed with MiSeq Reporter (n=3), Torrent Suite (n=4) or panel-adjusted analysis pipelines. The median coverage was 2353 reads (range 275-17096). Results are summarized in the table. Overall, most variants were detected by analysis pipelines. The median coverage was 2353 reads (range 275-17096).

<table>
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<th>Sample</th>
<th>Gene</th>
<th>DDS</th>
<th>Acc.</th>
<th>Mean UQD (bp)</th>
<th>CD $\ell$</th>
<th>Mean UQD (bp)</th>
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Conclusion: The interlaboratory comparative assessment of NGS for gene mutation analysis in myeloid neoplasms showed variability related to the analytical methods used. Augmenting the number of participating laboratories and improving the quality of reference DNA and panels could increase the comparability of results. Overall, the performances were satisfactory.

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METHYLATION AGE IN MPN PATIENTS AS A CORRELATE FOR DISEASE STATUS, ALLELE BURDEN AND THERAPEUTIC RESPONSE

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Background: Myeloproliferative Neoplasms (MPNs) result from genetic and epigenetic dysregulation. Epigenetic therapies, such as Vorinostat (SAHA, MK-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 CpGs within 3 genes, ASPA, ITGA2B, PDE4C enabled the determination of a functional age that reflected an individual’s actual age.

Aims: The aim of our study was 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 #2007-00356-49). 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 Polycythemia Vera (PV) patients (23 F/17 M) with a mean age of 62 years (range 29-81) were assessed. JAK2V617F was detected in 77.5% (n=31/40). Complete clinical response (CR) was achieved in 8 patients, partial (PR) in 17, and no response (NR) in 15 patients. MA was on average 8.3 years younger than CA (range -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 (-0.6 years vs -15.3, p=0.0122) and after 3 months therapy (-0.5 years vs -16.2, p=0.0072). Although the cohort size was small, within the ET group, NR compared to PR was associated with a younger MA after therapy (41.4 years vs 56.3, p=0.0156). Within PV, NR compared to PR was associated with a MA that was older than CA both before (+9.2 years vs -14.2 years, p=0.0346) and after therapy (+7.4 years vs -13.9, p=0.0347).

Suggested Conclusions: A link between MA and JAK2 mutant allele burden in MPN patients, suggesting that allele burden not only has a role in clinical phenotype and disease evolution but in the overall methylation landscape of the mutated cells. However, the role of MA with respect to therapeutic response needs to be clarified with further studies required to show its full impact.

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ELUCIDATING THE AGE INDUCED HEMATOPOIETIC CELL-INTRINSIC AND EXTRINSIC MECHANISMS IN MYELOPROLIFERATIVE NEOPLASMS INITIATION AND PROGRESSION

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Background: The number of detectable somatic mutations increase with age, but this increase is surpassed by the rise in the incidence of cancer in older people. The underlying mechanisms for this disparity remain to be elucidated. Myeloproliferative neoplasm (MPN) is an ideal malignancy model disease to study clonal hematopoiesis, disease initiation and progression during natural aging because the majority of the relevant mutations (such as JAK2 V617F) are catalogued, the disease evolves and progresses slowly allowing the collection of serial samples, and an inducible transgenic mouse models for the disease have been established. Nonetheless, the prevalent occurrence of such clonal events in aged individuals brings up the question, which age-associated cell-intrinsic and cell-extrinsic factors contribute to initiate hematologic malignancies and what are the rate limiting steps attributable for age-induced myeloid malignancies? We hypothesize that the induced different maligns provides a context that favors a selective process 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 JAK2-461D 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-intrinsic and cell-extrinsic factors that determine initiation and progression of MPN at 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 resulted in a cancer phenotype in aged wildtype mice. The mutation 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%), primary myelofibrosis (71% vs 57%), and high dipSS score (41% vs 14%). For those with BL anemia regardless of whether RBC transfusion-dependent (TD), PAC did not worsen hgb levels and the rate of clinical improvement in hgb was higher for pts in the PAC BID arm (25%) vs PAC QD (13%) or BAT (12%) arms (Table). For pts with CALE RBC transfusion independent (ITI) at BL (13% in RBC transfusion-dependency) was achieved at higher rates with PAC QD (19%) and PAC BID (22%) vs BAT (9%); 2 PAC and 0 BAT pts achieved RBC-Ti 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 8 weeks 16-8 for QD and BAT, respectively) and first 8 weeks of BAT (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 (27% vs 32%, respectively). In some instances of grade 3/4 anemia with PAC or BAT (Table) were in pts with BL hgb <10g/dL. Dose modifications or discontinuations due to anemia were uncommon (Table). No exposure-response relationship was evident for grade ≥2 TE anemia.

Summary/Conclusions: In pts with MF and BL thrombocytopenia, PAC treatment led to clinical improvement in hgb and reduction of RBC transfusion needs vs BAT. Serious anemia, and dose modifications due to anemia were uncommon. PAC provides a treatment option for pts with MF, including those with BL thrombocytopenia and anemia, for whom available options are limited.

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COMBINATION THERAPY OF POMALIDOMIDE PLUS RUXOLITINIB 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-0109 trial investigating pomalidomide (POM) in MF with cytopenia, anemia was improved in 14-29% of pts treated with 3.5-2mg POM once daily (QD) (Schlenk RF, Stegelmann F et al. Leukemia 2016).

Aims: To evaluate synergistic effects of POM plus ruxolitinib (RUX), we are currently investigating the combination therapy within the MPNSG-0212 trial (NCT01644110).

Methods: MPNSG-0212 is designed as multicenter, single-arm phase-IIb/1 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) in the first 16 weeks of PAC treatment (Figure 1).

Results: At study entry was 8.6 g/dL (range, 5.4-11.7); 11 pts (29%) were RBC-transfusion independent (ITI) at a median of 26.1 (95% CI 25.3-27.3) weeks. Of those 22, 19 pts remained on PAC treatment at the time of data cut-off, for 24 weeks of PAC treatment (Figure 1).

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PACRITINIB (PAC) VS BEST AVAILABLE THERAPY (BAT), IN PATIENTS WITH MYELOFIBROSIS (MF) AND BASELINE (BL) THROMBOCYTOPENIA: FOCUS ON RUXOLITINIB (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, FLI1, MPL, and CSF1R.

Aims: The safety and efficacy data from 38 pts are presented. Median age of the 38 pts was 66 (range, 38-83); 19 pts (50%) previously received hypomocytotoxic, RUX, EPO, POM, and/or corticosteroids. Median hemoglobin (Hb) level at study entry was 8.6 g/dL (range, 5.4-11.7); 11 pts (29%) were RBC-transfusion dependent. Median spleen size by ultrasound was 17.9 cm (range, 12.6-28). At baseline, 30 pts (79%) had constitutional symptoms, Mutations of JAK2, MPL and CSF1R present in 28 (74%), 3% (8%) and 7 (18%) pts, respectively; 26 (68%) were intermediate-2 risk and 9 (24%) high-risk according to the DipSS assay (Passamonti et al, Blood 2010). Median time on treatment was 12 cycles (range, 2-33). In total, 881 adverse events (AE) CTCAE 1-5 were recorded. Worsening of anemia within the first 6 cycles was the most frequent AE (32%) and serious AE (11%) followed by fatigue in 12 (32%). Treatment interruptions were rare. There were 29 serious AE (SAE) CTCAE 2-5 of most frequently, leukemic transformation (n=4), pneumonia (n=3), thoracic pain (n=3), abdominal pain (n=2), cardiac decompensation (n=2) and septic shock (n=2) occurred in 13 pts (34%) of which 5 were fatal (cardiac decompensation, pneumonia, sepsis, pulmonary edema, and cardiac decompensation). 3 pts died from neutropenia, toxicity, 3; fatigue, 3; and cardiac decompensation, 3 were considered study-related. 16 pts (42%) are currently on study treatment; 22 (58%) discontinued because of AE (n=6), withdrawal of consent (n=5), stable disease (SD) without objective response after 12 cycles (n=4), leukemia transformation (n=4) or death (n=13; 34%) responded with spleen reduction (n=9) or 22 g/dL Hb increase / RBC transfusion independence (n=4). Of note, mean Hb increased continuously from 8.7 g/dL at baseline to 9.2 g/dL at the end of cycle 12; 12 pts (31%) continued treatment beyond cycle 12 because of response or SD plus clinical benefit (Hb increase ≥2g/dL, prolongation of transfusion-free survival, and/or improvement of symptoms); 6 pts (16%) stayed on treatment for >24 cycles.

Summary/Conclusions: In our study in advanced MF, combination of POM plus RUX was feasible with an objective response rate of 34%. Approximately one third of pts was treated beyond cycle 12 due to sustained therapeutic benefit. Based on favorable safety profile and outcomes from our MPNSG-0212 trial, a step-wise increase of the POI dosage is included for the 2nd study cohort to further improve anemia response.

PT01
NATIONAL CONGRESS OF THE EUROPEAN HEMATOLOGY ASSOCIATION
P702
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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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.

Figure 1.

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PROGNOSTIC RISK MODELS FOR TRANSPLANT DECISION-MAKING IN MYELOFIBROSIS


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Background: MF is a clonal disorder of bone marrow progenitor cells characterized by ineffective hematopoiesis, extramedullary hematopoiesis, splenomegaly, and bone marrow fibrosis. The optimal treatment for MF is uncertain, with no standard of care established. Prior research has identified clinical and genetic risk factors associated with survival and transformation to acute leukemia, including primary and secondary MF. More recently, the World Health Organization (WHO) 2016 classification identified new entities and refined the definition of MF. Risk stratification models such as the IPSS-R, IPSS-MF, and IPSS Commercial model have been developed using different risk factors. However, the IPSS-R was developed using data from younger patients, and it is unknown whether it can accurately predict outcomes in elderly patients with MF.

Purpose: To develop and validate a risk-stratification model for elderly patients (≥65 years) with MF.

Methods: We conducted a retrospective study of patients with primary MF (≥65 years) treated at 10 Spanish centers from 2013 to 2016. Baseline characteristics, IPSS-R score, and outcomes were collected. The primary endpoint was overall survival (OS). Multivariate logistic regression analysis was performed to identify significant predictors. The model was validated using the Harrell method.

Results: A total of 421 patients were included in the analysis. Median age was 75 years (range, 65–92 years). A majority of patients were treated with ruxolitinib. The median follow-up time was 2 years. The IPSS-R score was a significant predictor of OS in the univariate analysis (p < 0.001). The multivariate analysis identified age (≥65 years), prior thrombocytopenia (platelet count <50 x 10^9/L), and prior transfusion as significant predictors of OS. The Harrell-Cox model showed good discrimination (c-index = 0.64) and calibration (Hosmer-Lemeshow test, p = 0.98).

Conclusion: The IPSS-R score is a significant predictor of OS in elderly patients with MF. A new risk-stratification model based on age, prior thrombocytopenia, and prior transfusion may improve the prognostic accuracy in this population.
Background: Accurate disease risk stratification is crucial for transplant decision making in patients with myelofibrosis (MF). However, several prognostic models are available, it is unknown if they are equivalent in the way they distribute patients into risk groups and in their discriminatory power to predict survival.

Aims: We have compared the performance of the International Prognostic Scoring System (IPSS), dynamic IPSS (DIPSS), DIPSS-plus, and Rumi’s score in a series of 544 MF patients aged 70 years or older 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 Falcifiladas negativas (GEMFIN). From January 2000 to January 2016, a total of 544 adult patients aged ≤ 70 years with primary MF (n=335) or secondary MF (n=209) had been included in the registry. Cases of the prefibrotic form of MF were not considered. Comparison of the relative power of each prognostic model to discriminate levels of risk was estimated by means of the Harrell’s concordance index (C-index) and the R^2 explained variation. All the statistical analyses were performed with IBM SPSS 22.0 and Stata 11.

Results: The allocation of 544 MF patients from diagnosis to disease risk categories of all classifications (and Rumi’s very low risk category). The projected survival for patients in the intermediate-1 group (intermediate in the Rumi’s score) and in the high risk group (very high risk in the Rumi’s score) was comparable in the four models. By contrast, the Rumi’s high risk group had a projected median survival of 9.2 years, whereas that of the intermediate-2 categories by the IPSS, DIPSS, and DIPSS-plus models was 6.6 years, 5.6 years, and 6.5 years, respectively. The number of patients in the intermediate-2 and high risk categories was smaller in the DIPSS than in the IPSS or the DIPSS-plus. Overall, the Rumi’s score yielded the highest power to discriminate between risk categories as assessed by the C-index and the R^2 explained variation. However, the IPSS and DIPSS-plus were the best models to discriminate between the intermediate-1 and intermediate-2 risk categories, which is the critical cut-off point for patient selection to transplant.

Summary/Conclusions: In our contemporary series of MF patients only the high risk patients were at high risk in 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.

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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 thrombocytosis (ET), hydroxy-carbamide (HD), has mutagenic properties and there is potential for leukemogenicity and secondary cancers with this agent. In the EXELS study, we have compared the performance of the International Prognostic Scoring System (IPSS), dynamic 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 assessed by the C-index and the R^2 explained variation. However, the IPSS and DIPSS-plus were the best models to discriminate between the intermediate-1 and intermediate-2 risk categories, which is the critical cut-off point for patient selection to transplant. Patient selection for transplantation is quite dependent on which prognostication model is used for disease risk stratification.

Summary/Conclusions: In our contemporary series of MF patients only the high risk patients were at high risk in 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.

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EPIDEMIOLOGY, OUTCOME AND RISK FACTORS FOR INFECTIOUS COMPLICATIONS IN MF PATIENTS RECEIVING RUXOLITINIB. A MULTICENTER STUDY ON 373 PATIENTS
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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: Overall, 373 pts received RUX between June 2011 and June 2016. At RUX start the clinical features were (median): age 68 years (27-89), ≥65y, 62%; male, 57%; Hb, 10.8g/dL (7-16.7); Hb <10g/dL, 40%; PLT, 246×10⁹/L (33-1887); PLT <100×10⁹/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%). JAK2V617F mutation was detected in 255 out of 313 evaluated pts (81%). Karyotype was unfavorable in 15 out of 203 evaluable pts (7%). Previous infectious complications were recorded in 31 pts (8%). After a median RUX exposure of 20 months (range, 1-56), 101 pts (27%) experienced 129 infectious events (grade 3, 33%), for an incidence rate of 14.9 cases for 100 pts/year. The rate of infections tended to decrease over time: 54% occurred within 6 months of therapy, 15% between 6 and 12 months, 9% between 12 and 18 months (p<0.001). Respiratory tract infections were more frequently observed (73 events, 57%). Cutaneous, urinary tract and gastrointestinal infectious events were diagnosed in 15%, 10% and 7% of cases, respectively. In 14 cases fever of unknown origin was recorded (Figure 1). Etiological agents were isolated in 14 cases (11%); bacteria in 9 cases (gram+ 56%, gram- 22%, C. difficile diarrhea 22%) and fungi in 2 cases (pulmonary aspergillosis and oesophageal candidiasis). Mycobacterium tuberculosis infection was isolated in 3 cases. Herpes-virus reactivations occurred in 12 cases (9%). No patients reactivated hepatitis B virus. At last follow-up, 88 pts (24%) have died, in 10 cases (11%) due to infectious complication. Among baseline features, age≥65 years at RUX start (p<0.001), previous infection (p=0.001), primary vs secondary MF (p=0.021) and high IPSS (p=0.029) significantly correlated with higher infectious risk. Notably, no differences were observed according presence of large (≥10cm) splenomegaly, higher (>20) total symptoms score, presence of cytopenias, Charlson comorbidity index (>2) and body mass index (>21 and >30). In multivariate analysis, PMF diagnosis (HR 1.6 [CI 95% 1.07-2.5], p<0.021), age≥65 years (HR 2.1 [CI 95% 1.3-3.3) and previous infection (HR 3 [CI 95% 1.7-5.4]) confirmed their negative prognostic association. Interestingly, RUX dosage, spleen response and hematological toxicities during treatment were not associated with infectious risk.

Summary/Conclusions: Infections occurred in around one-third of RUX-treated pts; the rate of infections tended to decrease over time, and were fatal in 11% of the cases. Advanced age, a previous infectious event and diagnosis of PMF seem to be the main contributors to infectious risk.
ET) chose watchful waiting to manage >25% of their pts at diagnosis; 22% of untreated pts had moderate to high (quartiles 3-4) overall symptom burden. Physicians primarily recommended treatment for pts experiencing severe symptoms (72% MF, 68% PV, 72% ET) or symptomatic splenomegaly (71% MF, 61% PV, 39% ET). PLB was mainly used to treat pts with PV. Of those who received PLB (n=155), 71% were very or somewhat satisfied; 25% were very dissatisfied and 4% felt that PLB had a negative impact on their QOL. Similarly, 37% of physicians felt that PLB had a negative impact on pt QOL; PLB alone was insufficient for disease control in 38% of pts. Pts stopped PLB because physician deemed it no longer necessary (62%), pts felt worse after treatment (10%), and visit frequency was inconvenient (8%). Physician-reported reasons for stopping PLB were that visit frequency was inconvenient (38%), pts felt worse after treatment (35%), and lack of intravenous access (33%). HU use was assessed in pts with PV or ET. Of those who received HU (PV, n=95; ET, n=145), 78% and 74%, respectively, continued to receive HU; 19% and 22% were dissatisfied with HU therapy. Main reasons for stopping HU were lack of efficacy (29% PV, 13% ET) and toxicity (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 discontinuation were key reasons for changing treatment.

Summary/Conclusions: Many pts with MPN 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 PEGINVERA STUDY
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Background: The outcome of patients with Philadelphia negative myeloproliferative neoplasms (MPN) who transform to acute leukemia is abysmal. There have been no advances in targeted therapy for this cohort of patients or individualized treatment based on genomic information. Furthermore, no large studies have investigated the impact of molecular profiling on clinical outcome in patients with accelerated or blast phase of MPN.

Aims: To describe the clinical outcomes of patients with MPN who transform to accelerated or blast phase and evaluate the impact of genomic alterations on outcomes.

Methods: Eligibility criteria included: Prior diagnosis of Philadelphia negative MPN according to WHO 2008 criteria; evidence of transformation to accelerated (10%-19% blasts in peripheral blood or bone marrow) or blast phase (>20% blasts) and seen at Princess Margaret Cancer Center between January 1998 and February 2017. The primary endpoint was overall survival (OS); defined as the time from transformation to death or last follow-up. Secondary endpoints included survival based on curative versus non-curative approach and treatment over time. In addition the impact of mutations will be correlated with clinical outcome and survival.

Results: One hundred and eighty-seven patients who transformed to accelerated or blast phase with a prior diagnosis of MPN were identified at our insti-
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 of response 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 ISM and more conventional patient-centric response endpoints.

Aims: To aide interpretation of this study’s prospectively declared primary endpoint via comparison with additional efficacy analyses based on a cohort restricted to the WHO classification of ISM and more conventional patient-centric response endpoints.

Methods: Randomized, placebo-controlled, phase 3 study that included 135 severely symptomatic ISM patients, including the subvariant smoldering systemic mastocytosis (71 masitinib, 64 placebo), 80% of whom satisfied the WHO classification.

Results: Masitinib showed a significant improvement over placebo according to its pre-specified primary endpoint (mITT population), with a cumulative response of 18.7% versus 7.4%, respectively, odds ratio (OR) of 3.6 [95%CI 1.2-10.8], P=0.008 (with re-randomization). This outcome was confirmed in the WHO patient subgroup: 17.8% versus 8.0%, respectively, OR=3.25 [0.97-10.88], P=0.0317. Computing the primary analysis (mITT) according to cumulative response per patient (GEE model) was also positive: 26.7% versus 12.8%, respectively, OR=2.48 [1.16-5.31], P=0.0212, as was analysis according to individual patient response (Pearson chi-square): 40.3% versus 24.2%, respectively, P=0.0062. Response (per patient) on all severe baseline symptoms for at least one visit was: 16.4% versus 11.6%, 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 other symptomatic treatments.

Other Non-malignant hematopoietic disorders

P709

MASITINIB FOR TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT SYSTEMIC MASTOCYTOSIS: ADDITIONAL EFFICACY ANALYSES FROM THE RANDOMIZED, PLACEBO-CONTROLLED, PHASE 3 STUDY AB06006

Masitinib for the Treatment of Severe Symptomatic Indolent Systemic Mastocytosis

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 of response 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 ISM and more conventional patient-centric response endpoints.

Aims: To aide interpretation of this study’s prospectively declared primary endpoint via comparison with additional efficacy analyses based on a cohort restricted to the WHO classification of ISM and more conventional patient-centric response endpoints.

Methods: Randomized, placebo-controlled, phase 3 study that included 135 severely symptomatic ISM patients, including the subvariant smoldering systemic mastocytosis (71 masitinib, 64 placebo), 80% of whom satisfied the WHO classification.

Results: Masitinib showed a significant improvement over placebo according to its pre-specified primary endpoint (mITT population), with a cumulative response of 18.7% versus 7.4%, respectively, odds ratio (OR) of 3.6 [95%CI 1.2-10.8], P=0.008 (with re-randomization). This outcome was confirmed in the WHO patient subgroup: 17.8% versus 8.0%, respectively, OR=3.25 [0.97-10.88], P=0.0317. Computing the primary analysis (mITT) according to cumulative response per patient (GEE model) was also positive: 26.7% versus 12.8%, respectively, OR=2.48 [1.16-5.31], P=0.0212, as was analysis according to individual patient response (Pearson chi-square): 40.3% versus 24.2%, respectively, P=0.0062. Response (per patient) on all severe baseline symptoms for at least one visit was: 16.4% versus 11.6%, 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 other symptomatic treatments.
had HLH solely attributed to malignancy (Figure 1).

The patients who developed hM-HLH with concomitant infection during chemotherapy show better survival than those who had HLH solely attributed to malignancy. Although poor outcome in some patients with M-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.

**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 M-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.

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**WHOLE-EXOME SEQUENCING IN CHILDREN WITH IMMUNE CYTOPEenia - THE APPLICABILITY AND CLINICAL IMPACT**

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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 due to (i) high altitude hypoxia, (ii) KIT (ISMs-), carry the D816V mutation in PB of ISM is associated with (ii) the presence of circulating tumor cells, (iii) multiple myeloid cell populations, KIT-mutated PB CD34+ HPC potentially contributing to disease dissemination already at very early stages.

P714
MONOALLELIC VARIANTS IN GENES RELATED TO FAMILIAL HEMOPHAGOCYTIC LHYSIOTICTOSIS: REPORT FROM THE ITALIAN REGISTRY
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Background: Hemophagocytic Lymphohistiocytosis (HLH) is a life-threatening disease of children and adults caused by an impaired cytotoxic function of NK and CTL cells leading to a potentially fatal hyperinflammatory condition. Biallelic mutations in genes involved in the cytotoxic pathway are responsible for the familial form of the disease (FHL). Monoallelic mutations in the FHL-related genes have been reported in association with HLH and other diseases but their role remains to be understood.

Aims: To describe clinical, functional and genetic features of patients referred to the Italian HLH Registry, harboring monoallelic mutations in FHL-related genes.

Methods: Patients with complete or partial HLH diagnostic criteria and monoallelic mutations in at least one of the FHL-related genes were selected from the Italian HLH Registry. Clinical data were collected by specific forms. Perform expres-

Summary/Conclusions: Of the 800 patients reported to the Registry, 54 (9%) were found to have monoallelic mutations in FHL-related genes. Their median age was 5 years (quartiles: 1.3, 3.13, 10 years). Twenty-nine of the 54 patients (54%) fulfilled at least 5 of the 8 diagnostic criteria: fever (n=49/52, 94%), splenomegaly (n=37/50, 74%), cytopenia (n=43/50, 86%), hypertriglyceridemia (n=28/47, 60%), hypofibrino-

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CHARACTERIZATION OF CD34+ HEMATOPOIETIC PRECURSORS IN INDOLENT SYSTEMIC MACROCYSTOSIS 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 periph-

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PRIMARY AND CONGENITAL ENRITHEROCRYSIS IN PEDIATRICS: THE EXPERIENCE OF ITALIAN CENTERS
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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 2 families a defect was identified (2 VHL, and 2 Hb variants). One Hb positive case was found sporadic. Most Hb variants were not symptomat, while all other familial cases had splenomegaly and vascular symptoms. Among non familial, non genetic cases, 5 children were affected by Down Syndrome; 4 children had severe renal or cerebral disease. In one 4 year old girl, with a polymorphic Hb variant, who presented with arterial hypertension, a small size ganglioneuroma was found after a 5yr follow-up. In 21 cases non causes could be identified. They were mostly male (n18); presented at adolescent age with advanced puberal status (n17); many were symptomat (6). Only one 9 year old girl was diagnosed with Polycythemia 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 patients severe vascular complications were observed (arterial thrombosis), even with Hct<45%.

Summary/Conclusions: This series shows the heterogeneity of Erythrocytosis as found in pediatrics. Extensive clinical and genetic analysis are required but still a large number of cases lack clear definitions. The usefulness of angiography and phlebotomy is not proved.

P716 NEUROLOGIC INVOLVEMENT IN EVANS SYNDROME AND CHRONIC HEMOLYTIC AUTOIMMUNE ANEMIA OF CHILDREN: DESCRIPTION, EVOLUTION AND GENETICS

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Background: Erythropoiesis, (i.e. increased levels of hemoglobin /hematocrit (Hb/Htc) >95percentile for age and sex), is rarely found in pediatric or adolescent age. Presence of familial cases, presentation at birth or presence of known mutations, as well as exclusion of secondary causes identifies primary (PE) or congenital secondary forms (CE). However, many cases still lack evident etiological definition (idiopathic E.). Moreover, natural course and treatment are still anecdotally reported.

Aims: Here we present our experience in a large and heterogeneous series of children with absolute erythrocytosis. The aims is to identify a possible clinical and diagnostic approach to children with erythrocytosis

Methods: All children with E. who lacked evidence of reactive origin were consecutively referred to our laboratory for molecular evaluation. Molecular analysis of the main involved genes (VHL, HIF2A, EPOR, JAK2, PHD2) was performed by allele specific PCR, PCR on direct DNA sequencing. 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.

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 symptomat, while all other familial cases had splenomegaly and vascular symptoms. Among non familial, non genetic cases, 5 children were affected by Down Syndrome; 4 children had severe renal or cerebral disease. In one 4 year old girl, with a polymorphic Hb variant, who presented with arterial hypertension, a small size ganglioneuroma was found after a 5yr follow-up. In 21 cases non causes could be identified. They were mostly male (n18); presented at adolescent age with advanced puberal status (n17); many were symptomat (6). Only one 9 year old girl was diagnosed with Polycythemia 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 patients severe vascular complications were observed (arterial thrombosis), even with Hct<45%.

Summary/Conclusions: This series shows the heterogeneity of Erythrocytosis as found in pediatrics. Extensive clinical and genetic analysis are required but still a large number of cases lack clear definitions. The usefulness of angiography and phlebotomy is not proved.
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.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA TREATMENT DURING PREGNANCY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a life-threatening disorder with a high risk of thrombosis. Targeted therapy radically changed the prognosis in PNH. Therefore issues of reproductive health in PNH patients are becoming very important. Recently the management of PNH during pregnancy has been challenging because of the high risk of maternal morbidity and frequent pregnancy loss. The combination of targeted therapy with eculizumab and anticoagulants made it possible not only to increase the survival rate, but also to improve the quality of life

Aims: We compared the pregnancy outcomes in PNH patients on eculizumab treatment and retrospective data of pregnancies on symptom 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.

Results: Clinical manifestations of hemolysis significantly regressed during eculizumab treatment: normalization of LDH was registered in 76.5% patients. Without eculizumab LDH level increased in all pregnant patients. No maternal death and thrombotic events have been observed. 42.9% of patients required a dose adjustment due to breakthrough hemolysis (a dose increase and/or more frequent use of eculizumab). Pregnancy complications were less frequent with eculizumab: abortion threat 35.3% vs 85.7%, fetal growth retardation syndrome 7.1% vs 21.4%, preeclampsia 5.9% vs 14.3%. Transfusion rate was higher without eculizumab (86.7% vs 41.2%). Pregnancies resulted in the birth in 100% patients exposed eculizumab and 42.9% on supportive treatment. Mean birth weight 2560 g (450-3550). Most of newborns (87.5%) are healthy, 83.3% of them received breastfeeding without complications both on eculizumab and without it.

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia, with platelet counts <100×10^9/L. Eltrombopag is an oral small-molecule nonpeptide thrombopoietin-receptor agonist that has shown to increase platelet counts. This is against the management of patients with chronic ITP (aged ≥1 year) who are refractory to other treatments (eg, corticosteroids, immunoglobulins). The recommended eltrombopag dose in patients with chronic ITP is 25mg once daily, OD (East Asians, 12.5mg OD or 25mg every other day) in pediatric aged ≥1-17 years at initiation, followed by dose adjustment to a maximum of 75mg OD based on platelet counts. REVIEU study was conducted in accordance with risk management plan in five European Union (EU) countries to document eltrombopag utilization patterns in real-world practice. Here, we report the eltrombopag utilization data on the subset of adult patients (aged ≥18 years) with ITP as primary diagnosis.

Aims: To evaluate the real-world data to determine drug utilization patterns among adult patients with ITP receiving eltrombopag within five EU countries.

Methods: REVIEU study was a multinational, multicenter, retrospective, medical chart review in patients with a documented past treatment with eltrombopag between the period immediately after first approval/launch in May 2010 and September 2014 (ie, dispensed at least once by the pharmacy and patient received at least one dose) for whatever reason. Patients who participated or were participating in a randomized eltrombopag clinical trial were excluded.

Table 1.

Results: Overall, 287 adult patients with ITP (chronic [≥12 months], 75.3%; persistent [3–12 months], 10.8%; acute [≥3 months], 13.6%; unknown [n=1]) were included, majority in Spain (n=128) followed by Italy (n=67), France (n=36), and Germany (n=27). Eltrombopag was the first treatment with no prior ITP therapies in 12 (4.2%) [acute, 10.3%; persistent, 6.5%; chronic, 2.8%] patients. A total of 99 (34.6%) patients received one prior therapy (corticosteroids, 79 [27.6%]; 128 (44.8%) patients received two prior therapies (corticosteroids + immunoglobulins, 114 [39.9%]; patients received three prior therapies (corticosteroids, immunoglobulins, and splenectomy). In total, the majority of patients received at least one prescription of corticosteroids (252, 88.1%) followed by immunoglobulins (180, 62.9%), and splenectomy (64, 22.4%) prior to eltrombopag initiation. Patients received an average daily dose of eltrombopag 45.6mg (chronic ITP, 43.1mg; persistent ITP, 43.5mg) during the study. Overall, dose changes were reported in 749 adult ITP prescriptions (down-titration, 53.7%; up-titration, 43.7%; no change in dose, 2.7%). 49.1% of dose changes were reported during the first 6 months of treatment (35% in first 3 months). The main reasons for dose change included: disease improvement (30.4%), no treatment response (26.8%) and others (27.1%). Disease improvement accounted for down-titration in 51.2% (206/402) and up-titration in 4.6% (15/327), and no treatment response for up-titration in 54.4% (178/327) and down-titration in 5.0% (20/402) of adult patients with ITP. 46.5% of adult patients with platelet counts by ITP disease phase, and by eltrombopag dose are reported in Table 1.

Summary/Conclusions: The majority of adult patients with ITP (75.3%) were diagnosed with chronic ITP, and were treated with eltrombopag as second-line or greater therapy after corticosteroids and immunoglobulins, in line with the approved indication. Eltrombopag was also prescribed in 24.4% of adult patients with acute and persistent ITP. The starting dose followed the summary of product characteristics (SmPC) recommendations in the majority of cases and dose modifications were generally according to platelet counts. Data from REVIEU study have shown that eltrombopag use in the real world setting is largely consistent with the EU label and is considered part of ITP medical practices.

**Table 1.** Proportion of patients with platelet counts by ITP disease phase, and by eltrombopag dose

**Table 2.** Proportion of patients with platelet counts by ITP disease phase, and by eltrombopag dose
Results: of mean fluorescence intensity (MFI) compared to that of a control. Modified antigen capture ELISA test (MACE) was performed to assess the specificity of platelet autoantibodies. Western blot (FXI, FXII) and HPLC (trans-sialidase) were used to analyze their ability to induce desialylation in normal platelets. Analysis of desialylation included the use of Agglutinin I (RCA-1), a lectin that binds to galactose residues underlying sialic acid. Patients' sera was also incubated with normal human platelets to analyze surface expression of platelet glycoprotein (GP) IIb, and the activation marker CD63.

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 (GPIb) IIb, and the activation marker CD63 were examined by flow cytometry (FC) analysis, as well as desialylation of platelet membrane proteins using fluorescein-conjugated Ricinus Communis Agglutinin I (RCA-1), a lectin that binds to galactose residues underlying sialic acid. 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-sialidase).

Aims: The aim of this study was to analyze the biological features of ITP patients refractory to conventional therapies.

Summary/Conclusions: This study shows a significant higher platelet desialylation in ITP patients who are non-responders to conventional therapies, particularly if they are also refractory to TPO-RA. According to a previous study (1), these results seem to be associated to platelet activation mediated by anti-platelet specific antibodies.


Table 1.

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Background: A previous study has suggested a mechanism of Fcy receptors (FcyR)-independent platelet clearance in immune thrombocytopenia (ITP) (1). Antibody-directed platelet desialylation may lead to platelet clearance in the liver via hepatic Ashwell–Morell receptors, providing a potential explanation for refractoriness to classical therapies (steroid, IVIG and splenectomy).

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| 1. Fuentes, J., B. Bermejo, J. M. Bastida, J. Corral, V. Vicente, M. L. Lozano, Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, Murcia, 2 Hospital de Jerez, Jerez de la Frontera, 3 Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, Murcia, 4 Hospital Universitario Reina Sofia, Córdoba, 5 Hospital Obispo Polanco, Teruel, 6 Hospital De Dénia, Denia, 7 Hospital Infantil Cristina, Badajoz, 8 Hospital San Pedro de Alcántara, Cáceres, 9 Hospital Universitario Salamanca, IBSaL, Salamanca, 10 Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, Grupo de investigación CD15/00055 del CIBERER, ISCIII, Murcia, Spain |

Background: A previous study has suggested a mechanism of Fcy receptors (FcyR)-independent platelet clearance in immune thrombocytopenia (ITP) (1). Antibody-directed platelet desialylation may lead to platelet clearance in the liver via hepatic Ashwell–Morell receptors, providing a potential explanation for refractoriness to classical therapies (steroid, IVIG and splenectomy).

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 CD63 were examined by flow cytometry (FC) analysis, as well as desialylation of platelet membrane proteins using fluorescein-conjugated Ricinus Communis Agglutinin I (RCA-1), a lectin that binds to galactose residues underlying sialic acid. 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-sialidase).

Aims: The aim of this study was to analyze the biological features of ITP patients refractory to conventional therapies.

Summary/Conclusions: This study shows a significant higher platelet desialylation in ITP patients who are non-responders to conventional therapies, particularly if they are also refractory to TPO-RA. According to a previous study (1), these results seem to be associated to platelet activation mediated by anti-platelet specific antibodies.


Table 1.
Results: A total of 546 pts received either R or E between Dec 2009 and Dec 2015. Of these, 106 (19.4%) underwent TPO-RA switch. Table 1 summarizes outcome after switch. Overall 69/106 (65%) of pts achieved, regained or maintained response upon switching. Either one TPO-RA switch 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 was lower responders (p=0.020): re-exposure to a switching back 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. 1 study with thrombocytopenic cytopenia patients presented 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 back 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 leading to a lower response rate in 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.

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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 thrombopoietin receptor agonist approved for treatment of previously treated patients (pts; eg corticosteroids, immunoglobulins) with cITP aged ≥1 yr. The EXTEND study, a global, open-label, extension study of pts with cITP who received EPAG or placebo in prior EPAG studies, evaluated long-term safety and tolerability of EPAG. In EXTEND, 19 (6.3%) pts receiving EPAG experienced a total of 24 thromboembolic events (TEEs; Sarpatwari et al. Haematologica 2010;95:1167-75), which is similar to TEE incidence in cITP pts receiving romiplostim (Kuter et al. Br J Haematol 2013;161:411–23) and to one estimate in the general cITP population (Sarpatwari et al. Haematologica 2010;95:1167-75).

Aims: To describe management and outcomes of TEEs occurring during EPAG treatment in the EXTEND study.

Methods: Adult pts with cITP received EPAG starting at 50mg/day, with titration to 25–75mg per day or less as required, based on individual platelet count responses (target range ≥50–200×10^9/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 AE, including investigator-reported TEEs.

Results: 302 pts were enrolled and received ≥1 EPAG dose: 67% female; 38% of pts received ≥1 TPO-RA (1st TPO-RA exposure duration ≥2.4 yrs (range, 2 days to 8.8 yrs) and mean daily dose was 50.2 (range, 1-75)mg/day). Overall, 259/302 (86%) pts achieved platelet counts of ≥50×10^9/L (≥150×10^9/L in nine; 6 pts experienced the TEE at or shortly after achieving ≥150×10^9/L). TEE risk factors increased with platelet count <10×10^9/L (P=0.027, OR=1.865, 95% CI 1.074-3.238). Compared to severe (non-ICH) bleeding, Cox regression analysis was used to estimate rate ratios (RR) for no remission and mortality.

Results: Among 517 patients with ITP, 10 (1.9%) presented intracerebral hemorrhage (ICH) and 74 (14.3%) presented severe (non-ICH) bleeding during ITP. According to multivariate analysis, risk of severe bleeding in patients was increased with platelet count <10×10^9/L (P=0.001, OR=1.682, 95% CI 1.271-2.234), female patients (P=0.010, OR=2.148, 95% CI 1.200-3.844), complication 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 similarly 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). Total relative risk rate (CRHR) 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%, 238/340, χ²=0.014, 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=4.543, 95% CI, 1.317-15.688).

Summary/Conclusions: Platelet count <10 000/μL, 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.

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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) and bone marrow stromal cells (BMSCs), that are derived from the bone marrow (BM) microenvironment regulates MKs maturation and thrombopoiesis. We recently reported that the impaired BM EPCs, which could be quantitatively and functionally improved by atorvastatin in vitro, induced the occurrence of poor graft function following allo-transplantation (Blood, 2016, 128:2988-2999). However, little is known about the functional role of BM EPCs and how to improve impaired BM EPCs in patients with corticosteroid-resistant ITP.

Aims: To determine whether quantitative and/or functional abnormalities of BM EPCs are involved in the occurrence of corticosteroid-resistant ITP. Moreover, to investigate the effects of atorvastatin and N-Acetyl-L-cysteine (NAC, a ROS scavenger) on the number and function of cultivated BM EPCs derived from patients with corticosteroid-resistant ITP and its underlying molecular mechanisms. Finally, to evaluate the efficacy and safety of atorvastatin and NAC to adult patients with corticosteroid-resistant ITP.

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 patients with corticosteroid-resistant ITP and its underlying molecular mechanisms. In the study.

Results: Human bone marrow EPCs were demonstrated as the spindle shape and the similarity expression of CD34, VEGFR2 and CD133 at day 7 of cultivation among all groups of subjects and dysfunctional BM EPCs, which were characterized by impaired proliferation, migration, angiogenesis, and higher levels of ROS and apoptosis, were revealed in corticosteroid-resistant ITP patients compared to those in newly diagnosed ITP. Activation of p-P38 was detected in BM EPCs from corticosteroid-resistant ITP patients. Furthermore, the number and function of BM EPCs derived from corticosteroid-resistant ITP patients were enhanced by atorvastatin or NAC treatment in vitro through down-regulation of the p38 mitogen-activated protein kinase (MAPK) pathway. In the single-center pilot study, a total of 12 corticosteroid-resistant ITP patients were recruited to receive either the combination of atorvastatin and NAC or alone. AID and CR/R and OR results were (3/12), 41.7% (5/12) and 66.7% (8/12), respectively. In patients who achieved CR and R, the median (range) TTR was 24 days (7-51 days), with no apparent adverse events.

Summary/Conclusions: The number and the function of BM EPCs were improved by atorvastatin in vitro 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.

P726

PLATELET DESIALYLATION: A NOVEL MECHANISM AND A THERAPEUTIC TARGET IN THROMBOCYTOPENIA DURING SEPSIS: AN OPEN-LABEL, MULTICENTER, RANDOMIZED CONTROLLED TRIAL

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Background: Sepsis is a systemic, deleterious host response to infection leading to severe sepsis, and possibly septic shock as defined by the Surviving Sepsis Campaign guidelines. Thrombocytopenia is a common finding in sepsis. Studies in murine models suggested that platelet desialylation was an important mechanism of thrombocytopenia during sepsis. Desialylation-induced platelet removal could possibly be circumvented by adding sialidase inhibitors during sepsis. Oseltamivir, also known as Tamiflu, is a viral sialidase inhibitor that prevents the release of progeny virions. Several studies suggested the feasibility that oseltamivir can be used for the treatment of infection-associated thrombocytopenia.

Aims: To determine whether thrombocytopenia is associated with increased platelet desialylation in septic patients, and whether oseltamivir is an effective treatment to increase platelet counts in severe sepsis.

Methods: We first performed a prospective, multicenter, observational study that enrolled septic patients with or without thrombocytopenia to determine the association between platelet desialylation and thrombocytopenia in patients with sepsis. Next, we conducted an open-label, randomized controlled trial in which the patients who had severe sepsis with thrombocytopenia (platelet counts ≤50 × 10^9/L) were randomly assigned to receive antimicrobial therapy alone (control group) or antimicrobial therapy plus oseltamivir (oseltamivir group). The study flowchart is shown in Fig. 1. Both groups received appropriate antimicrobial agents and standard medical support based on the guidelines issued by the Surviving Sepsis Campaign. The oseltamivir group additionally received 5 full days of oseltamivir therapy. The oseltamivir was administered orally or through a feeding tube at a dose of 75 mg once every 12 hours. Time from randomization to the administration of oseltamivir was less than 24 hours. The antimicrobial agents were continuously administered until 3 days after the resolution of the physiological abnormalities related to the systemic inflammatory response syndrome (SIRS). The primary outcomes were platelet desialylation level at study entry, and overall platelet response rate within 14 days post-randomization. Secondary outcomes included platelet recovery time, the occurrence of bleeding events, and the amount of platelets transfused within 14 days post-randomization. The percentages of platelets positive for Ricinus communis agglutinin I (RCA-I), Erythrina cristagalli lectin (ECL) or Succinyl Triticum vulgare lectin (sWGA) analyzed by flow cytometry represented the levels of platelet desialylation. Platelet response was defined as platelet counts returning to or above 100 × 10^9/L. Platelet recovery time was calculated as the date of normalization to the date when platelet counts were >100 × 10^9/L. Written informed consents were obtained from the study participants prior to inclusion in the study.

Figure 1.
Results: The platelet desialylation levels increased significantly in the 127 septic patients with thrombocytopenia compared to the 134 patients without thrombocytopenia. A platelet response was achieved in 45 of the 54 patients in the oseltamivir group (83.3%) compared with 34 of the 52 patients in the control group (65.4%; P=0.045). The median platelet recovery time was 5 days (interquartile range 4-6) in the oseltamivir group compared with 7 days (interquartile range 5-10) in the control group (P=0.003). The amount of platelets transfused decreased significantly in the oseltamivir group compared to the control group (P=0.044). The multivariate analysis by Cox proportional hazards models showed that the Sequential Organ Failure Assessment (SOFA) score and platelet recovery time were independent indicators of oseltamivir therapy.

Summary/Conclusions: Thrombocytopenia was associated with increased platelet desialylation in septic patients. The addition of oseltamivir could significantly increase the platelet response rate, shorten platelet recovery time and reduce platelet transfusion. Chinese Clinical Trial Registry, ChiCTR-IPR-1600542.

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SAFETY AND EFFICACY OF LONG-TERM OPEN-LABEL DOSING OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)
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Background: Children with ITP for ≥6 months who completed a romiplostim phase 1/2 or phase 3 parent study could enroll in this open label long term extension study

Aims: To evaluate the safety and efficacy of long-term romiplostim in children with ITP.

Methods: Patients enrolled at 28 sites in the US, Canada, Spain, and Australia. All patients received SC romiplostim once weekly. The initial dose was the final dose from the parent study or 1 µg/kg for patients previously receiving placebo; dose was then adjusted from 1-10 µg/kg to target platelet counts of 50−200×10^9/L. Incidence of adverse events (AEs) was the primary endpoint.

Results: As of 24 Feb 2016, 66 patients entered this study; 65 received romiplostim for up to 6.2 years. At baseline, median (min–max) age was 11 (3–18) years; 56% were female; 61% were white, 14% African American, 14% Hispanic/Latino, 9% Asian, and 3% other; 9.1% had prior splenectomy. Median (min–max) baseline platelet count was 27.5 (2–458)×10^9/L. Median (min–max) treatment duration was 100 (5–321) weeks. Median (min–max) average weekly romiplostim dose was 4.8 (0.1–10.0) µg/kg, which included escalation to a stable dose. After ~week 200 (n ≤8 patients), the median dose was observed to fluctuate. All 65 patients received their doses per protocol >90% of the time; 18 patients missed ≥1 dose due to noncompliance for a total of 41 times. Reasons for discontinuing treatment (n=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 (gastrointestinal 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.

Figure 1.

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Quality of life, palliative care, ethics and health economics 2

P728 IMPACT OF VENETOCLAX ON THE QUALITY OF LIFE OF CLL PATIENTS RELAPSED/REFRACTORY TO B-CELL RECEPTOR (BCR) SIGNALING PATHWAY INHIBITOR TREATMENT

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Background: The prognosis for patients with CLL after B-Cell Receptor inhibitor (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 withibrutinib and/or idelalisib, have relapsed on treatment, or experienced progression after discontinuation of either agent. Patients are to receive VEN monotherapy for up to two years, or until discontinuation due to disease progression, unacceptable toxicity, or any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16, which were assessed at Baseline (BL), Week 24, and every 12 weeks thereafter. Mean change from BL to each assessment through Week 48 are reported here. Clinical relevance was based on minimum important difference (MID) of values from BL to each assessment. A change of 5-10 points is considered a “small” change on the EORTC-QLQ-C30. The lower bound of 5 points was used for MID acceptance on both measures.

Results: Thirty-five patients 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. Results from Baseline to Week 48 in VEN-Treated Patients

<table>
<thead>
<tr>
<th>Measure</th>
<th>BL to Week 48</th>
<th>Mean Change</th>
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<tr>
<td>EORTC QLQ-C30 Global Health</td>
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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 personal- ity 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 61% of pts. A change of 11% of pts from feeling insecure and not able to sustain 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 redundant and undecided 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 (CLL). Compared to the originator rituximab, significant price reductions are projected offering a more affordable treatment option for CLL patients across Europe.

Aims: To assess the budget impact of the introduction of CT-P10 into the treatment of CLL in the 28 EU member states. Moreover, we provide an estimation for the number of additional CLL patients that can be treated with CT-P10 from the cost savings.

Methods: A budget impact analysis was performed to evaluate the one-year cost outcomes under two scenarios with and without the availability of CT-P10. The budget impact was calculated as the difference in costs between the two scenarios. For the major European markets, five-year cost savings were also estimated. Market uptake of CT-P10 was assumed to be 30%. A third party payer’s perspective was adopted, and only drug costs were considered. Based on expert opinion, it was assumed that when CT-P10 is entering the market it will be at 50-70% of the official list price of originator rituximab in each country. Costs of administration and monitoring were not incorporated in the calculations, as it can be assumed that these are equal for the reference product and CT-P10. The initial number of patients treated with rituximab was estimated from IMS sales data on total annual consumption of originator rituximab in 2016. Other model parameters such as patients’ average body surface area and treatment rate of rituximab among CLL patients, were derived from the published literature. A one-way sensitivity analysis was undertaken to test the robustness of model assumptions.

Results: Over a one year time horizon, the cumulative budget impact of adopting CT-P10 is estimated to be €17.80 million in the 28 EU member states (30% discount in drug prices compared to the originator rituximab). Countries responsible for the majority of the cost savings are Germany (€4.06 million), Italy (€3.15 million), France (€2.41 million), Spain (€1.50 million), the UK (€1.34 million), Poland (€0.80 million), Austria (€0.66 million), the Netherlands (€0.59 million), Finland (€0.49 million) and Sweden (€0.43 million). If the cost savings were used to treat additional CLL patients with CT-P10, a total of 1,624 patients could be treated annually throughout Europe. The potential economic savings are in a direct correlation with the price and market uptake of CT-P10. Applying a 40% and 50% discount in drug prices compared to the originator rituximab, cost savings are projected to €23.73 and €29.67 million, from which further

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Madrid, Spain, June 22 – 25, 2017
**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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**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 determine the acceptability of various methods 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 perceived acceptability of remote monitoring following the survey. All patients preferred to continue to see a doctor if they had any concerns after TC with a nurse. Patients were perceived to have more expertise than nurses and this influenced preference for healthcare resourcing rather than them personally. Interpretation of blood and bone as either hyper-CVAD or DFCI replacing chemotherapy with treatment consultations (TC) replacing open coding by a doctor, physiotherapist and psychologist. 24% required hospitalization after the initial assessment, the clinical information obtained included: physical, mental, social and psychological well-being, with TC perceived as less burdensome. Patients acknowledged reduced needs during remission compared to treatment phase and felt TC would benefit redistribution of consultant time for patients on active therapy. Some suggested this service change would be beneficial for healthcare resourcing rather than them personally. Interpretation of blood results by clinicians was regarded as central to monitoring disease, and for some who were unaware of clinical symptoms, the only way a relapse would be detected. General preference was for bloods to be done locally, leading to concerns about availability of results for TC. Patients were unsure how to monitor their own MM, hence valued the knowledge of their medical team. Doctors were perceived to have more expertise than nurses and this influenced preferences regarding who undertook TC. As a result, patients sought reassurance they could see a doctor if they had any concerns after TC with a nurse. Patients valued continuity of care under the centre where they were treated due to prior positive experience and the importance of being seen at a tertiary centre renowned for its expertise in MM. This influenced acceptability of TC as long as they remained under the centre’s care with preference for continuity of staff involved. Whilst TC was acceptable for patients in remission, some were concerned about how relapse would be managed and expressed preference for FTF when being told they had relapsed.

**Summary/Conclusions:** Nurse led TCs are an acceptable alternative to FTF consultations for monitoring patients in remission from MM. Design of healthcare systems incorporating TCs need to have robust systems for accessing blood test results, for managing relapse, ready access to doctors and reassurance about the competence and knowledge of practitioners involved.

**P732**

**COST-EFFECTIVENESS OF RITUXIMAB IN ADDITION TO STANDARD OF CARE CHEMOTHERAPY FOR ADULT PATIENTS WITH ACUTE LYMPHOCYTIC LEUKEMIA**

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**Background:** In The Group for Research on Adult Acute Lymphoblastic Leukemia (GROALL-P), 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. We attempted to examine this question in the context of the Canadian publicly funded health care system.

**Aims:** To determine the economic impact in Canada of the addition of rituximab to standard of care (SOC) chemotherapy vs SOC alone in newly diagnosed CD20+ Ph- BCP-ALL.

**Methods:** Standard of care consisted of the two most widely used chemother- apy regimens for adults with ALL in Canada: hyper-CVAD or the Dana Farber Cancer Institute (DFCI) ALL consortium. A decision analytic model included all model inputs. 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 resulting mean Incremental Cost-Effectiveness Ratio (ICER) was C$40,505/QALY. At a willingness-to-pay threshold of C$100,000/QALY, the probability of being cost-effective was 96%. Decision outcomes were robust to the probabilistic and deterministic sensitivity analyses, including the SOC backbone as either hyper-CVAD or DFCI.

**Summary/Conclusions:** For adults with CD20+ Ph- BCP-ALL, rituximab in addition to SOC is a cost-effective intervention compared to SOC alone, from a Canadian public payer perspective. Rituximab is associated with increased life years and increased QALYs at a reasonable incremental cost.
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 10.9 years for controls). In a conditional logistic regression analysis adjusted for birth weight, a significantly increased leukemia risk (OR=4.75, 95% CI 1.55, 14.5) was found for any CT examination (one or more) at least two years prior to leukemia diagnosis. When comparing one CT examination and two or more CT examinations with no examinations the ORs were respectively 2.78 (95% CI 0.73, 10.5) and 16.9 (95% CI 1.81, 150).

**Summary/Conclusions:** In our preliminary analyses we observed a substantial increase in childhood leukemia risk related to pediatric CT scans. The risk estimates are materially higher than in two earlier studies \(^1\)-\(^2\) and need to be interpreted with caution. We will seek to estimate radiation doses to the red bone marrow, based on limited data available on CT examinations (body part and examination type).

**References**

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**P734**

**RADIATION EXPOSURE FROM CT IMAGING AND CHILDHOOD LEUKEMIA: A NATIONWIDE CASE-CONTROL STUDY**

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**Background:** Pediatric CT imaging offers significant benefits in clinical practice. However, children are more sensitive to carcinogenic effects of ionizing radiation than adults and red bone marrow is especially radiosensitive tissue type. The risk estimates of low doses of ionizing radiation are mainly \(^1\)-\(^2\) based on extrapolated results of studies done with substantially higher radiation doses and there exists a need to assess the risks of low doses with a more direct approach.

**Aims:** We assessed the leukemia risk in children after computed tomography imaging studies with high-quality Finnish register data and data from hospital databases.

**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=1093) 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 10.9 years for controls). In a conditional logistic regression analysis adjusted for birth weight, a significantly increased leukemia risk (OR=4.75, 95% CI 1.55, 14.5) was found for any CT examination (one or more) at least two years prior to leukemia diagnosis. When comparing one CT examination and two or more CT examinations with no examinations the ORs were respectively 2.78 (95% CI 0.73, 10.5) and 16.9 (95% CI 1.81, 150).

**Summary/Conclusions:** In our preliminary analyses we observed a substantial increase in childhood leukemia risk related to pediatric CT scans. The risk estimates are materially higher than in two earlier studies \(^1\)-\(^2\) and need to be interpreted with caution. We will seek to estimate radiation doses to the red bone marrow, based on limited data available on CT examinations (body part and examination type).

**References**

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**P735**

**HEALTHCARE RESOURCE UTILIZATION WITH IXAZOMIB OR PLACEBO PLUS LENALIDOMIDE-DEXAMETHASONE IN THE RANDOMIZED, DOUBLE-BLIND, PHASE 3 TOURMALINE-MM1 STUDY IN RELAPSED/ REFRACTORY MULTIPLE MYELOMA (RRMM)**

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**Background:** Treatment paradigms for RRMM have evolved in recent years with the approvals of multiple novel agents and evidence of benefits for using triplet vs doublet therapy and continuous treatment until progression. With more complex regimens and longer treatment duration, costs of treatment and healthcare resource utilization (HRU) are expected to increase, with IV agents having a greater impact on treatment burden than oral agents. The oral proteasome inhibitor ixazomib is approved in the US, EU, and multiple countries worldwide, in combination with lenalidomide-dexamethasone (Rd), for the treatment of RRMM patients (pts) following at least 1 prior therapy. Approval was based on the phase 3 TOURMALINE-MM1 study of ixazomib-Rd vs placebo-Rd, which demonstrated significantly improved progression-free survival (PFS; median 20.6 vs 14.7 months, HR 0.74) with ixazomib-Rd, with limited additional toxicity and no adverse impact on patient-reported quality of life (QoL; Moreau et al, N Engl J Med 2016).

**Aims:** HRU was an exploratory endpoint of the TOURMALINE-MM1 trial. The aim of this analysis was to compare HRU with ixazomib-Rd vs placebo-Rd, incorporating all non-protocol additional medical care encounters such as inpatient and outpatient admissions and their duration, as well as time lost from work or other activities by pts and their caregivers.

**Methods:** 722 RRMM pts with 1-3 prior lines of therapy received ixazomib 4mg (n=360) or matching placebo (n=362) on days 1, 8, and 15, plus lenalidomide 25mg on days 1-21 and dexamethasone 40mg on days 1, 8, 15, and 22, in 28-day cycles until disease progression or unacceptable toxicity. The primary endpoint was PFS. HRU was assessed on day 1 of each cycle prior to treatment and every 4/12 weeks during PFS/overall survival follow-up. After a median follow-up of ~23 months, pts had received a median of 17 (range 1-34) and 15 (1-34) cycles of ixazomib-Rd and placebo-Rd, respectively; HRU data are reported from this analysis time point.

**Table 1.**

<table>
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The ORs were 2.78 (95% CI 1.91, 150)


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Results: Overall, 152 (42%) pts on the ixazomib-Rd arm had 316 hospitalization events, compared to 156 (43%) pts (335 events) on the placebo-Rd arm. Exposure-adjusted hospitalization rates (0.530 and 0.564 per pt-year [ppy], respectively) and mean length of stay (10 and 10.8 days) were similar between the ixazomib-Rd and placebo-Rd arms (Table 1). Rates of outpatient visits were also similar between arms; 217 (60%) pts on the ixazomib-Rd arm had 197 (median 4) compared to 198 (55%) pts and 194 visits (median 5) on the placebo-Rd arm. Exposure-adjusted outpatient visit rates were 3.305 and 3.355 ppy, respectively (Table 1). On the ixazomib-Rd arm, 46 (13%) pts missed a total of 527 (median 7) days of work or other activity, compared to 51 (14%) pts and 580 (median 8) days on the placebo-Rd arm. Similarly, 16 (4%) pts caregivers missed 428 (median 5) days of work or other activity on the ixazomib-Rd arm, compared to 24 (7%) pts’ caregivers and 110 (median 4) days on the placebo-Rd arm.

Summary/Conclusions: The ixazomib-Rd triplet regimen did not add to the HRU burden compared to the placebo-Rd doublet, while prolonging PFS. The improvement was consistent with the limited additional toxicity burden and the reported lack of an adverse impact on QoL with ixazomib-Rd. In contrast to findings reported for injected agents (Armoiry et al, J Clin Pharm Ther 2011; Gaultney et al, J Clin Pharm Ther 2013; Baz et al, Support Care Cancer 2015), this all-oral triplet regimen did not increase time lost from work, caregiver burden, or the number of inpatient/outpatient visits.

P736 MANAGEMENT, ECONOMIC AND SOCIAL IMPACT OF SUB-CUTANEOUS RITUXIMAB ADMINISTRATION IN LYMPHOPROLIFERATIVE MALIGNANCIES
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Background: Lymphoproliferative disorders (LD) represent a major burden of hematologic malignancies generally treated and followed in Hematological Day Hospital (DH). Rituximab (antiCD20) and Follicular Lymphoma (FL) are among the most frequent LD treated with chemo-immunotherapies. These therapies are time consuming and costing and may affect the Quality of Life (QoL) of these patients because of their prolonged stay in DH.

Aims: To evaluate, in patients with DLBCL and FL, the economic and social impact of subcutaneous Rituximab administration compared to the intravenous formulation. During one week we evaluate in 40 patients the time of intravenous and subcutaneous administration, the type of treatment (rituximab combined or subsequent to chemotherapy, or in monotherapy) and the time required by the pharmacy to prepare each formulation. Moreover, we collected and analyzed data about patients’ time expenditure in DH until discharge. Collected data have been categorized as follows: time and human resource employment; drug waste; safety for patient. In order to measure the impact of QoL of subcutaneous formulation, we administered a questionnaire of satisfaction to the patients affected by DLBCL and FL and their 40 caregivers. Furthermore, we evaluated its role in the optimization of DH management in terms of time, professionals and economic expenditure and in improving patients’ safety.

Results: Among the 40 patients, 55% were affected by DLBCL and 45% by FL, 64% were males and 36% females; as for age 68% were over 60 years. The questionnaire examined patient’s emotions and perceptions during rituximab administration (anxiety, fear and pain), time required for infusion and interference with daily activities. Overall, 98% of interviewed patients preferred the subcutaneous administration because less scared by this formulation and because of the lower waste of time. Among the 40 interviewed caregivers 68% were workers. They considered advantageous the subcutaneous formulation because less scared by this formulation and because of the lower waste of time. How-
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 skin.

Aims: To assess the impact of hematopoietic stem cell transplantation (HSCT) for isolated extramedullary relapse (EMR) in children. 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. EMR was defined according to the Rome criteria for legal guardians. 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). Among patients transplanted with disease were included in the analysis. If a matched familiar (MF) 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 if both HSCT with a very long follow up. Comparison with those treated from 1995 to 2000.

Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to tests, 14 to mediastinum, 11 to CNS + other sites and 18 to other organs. Thirty one percent of children experienced a late relapse, 34.5% an early relapse, 31% a very early relapse, for 3.5% the time of relapse is not known. Ninety-seven patients underwent auto HSCT, 79 MFD HSCT, 75 MUD HSCT and 30 Haplo HSCT. At transplantation 72.6% of children were in CR2, 21.0% in CR>2 and 6.4% were not in remission Total body irradiation (TBI) (35 days from SCT (range, 11-254 days). Patients were heavily pre-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. 1.02-12, p=0.05); not receiving a prior regimen containing cyclophosphamide-TBI, fludarabine- melphalan-thiotepa, or busulfan-fludarabine-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 35 days from SCT (range, 10-45 days). Patients receiving IO were included, including 18 who had a prior aggressive 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. 1.02-12, 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.
Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal abnormality of hematopoietic stem cell leading to lack of phosphatidylinositol glycolipid C (PIgC) to prevent further complement activation. PNH results in severe hemolysis and can cause other complications such as thrombosis and organ dysfunction. Treatment options are limited, with allo-HCT being the only curative treatment, although outcomes are poor due to GVHD and TRM.

**Aims:** The aim of this study was to evaluate the safety and effectiveness of allo-HCT in PNH.

**Methods:** We report 41 allo-HCTs: 37 from MUD and 4 from MRD performed for 15% in 2004-2016. Median age of recipients was 20-62 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 gi/m2 plus fludarabine 5x30mg/m2 (31 pts) or treosulfan 2×14 gi/m2 plus cyclophosphamide 4x10gi/m2 (10 pts).バンタムのGVHD prophylaxis consisted of cyclosporine-A, methotrexate and pre-transplant ATG in MUD-HCT. 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 6.3x10XCI/kg. 5, 7.1x101CDS4+cells/kg, 24.7x109CD3+cells/kg (complemented in all pts with median 91-20 days of absolute agranulocytosis <0.1 G/l). Median number of transfused RBC and platelets units was 9(0-16) and 8(2-18).

**Results:** Of 100 HSCT pts with a confirmed diagnosis of PNH/OSOS and receiving 1x dose of DF, 264 (26.4%) had late-onset VOD/SOS, of whom 139 (52.7%) had MOD. By group, 95/264 (36.0%) were pediatric (aged ≤18 years; 5/06 [53.7%] with MOD) and 169/264 (64.0%) were adults (aged ≥16 years; 88/169 [52.1%] with MOD). Kaplan-Meier estimated survival at Day +100 (Figure) was 52.8% (95% CI, 45.6–58.7%) across all HSCT pts and 43.9% (95% CI, 35.4–52.0%) for pts with MOD; for pediatric pts, this was 60.4% (95% CI, 49.6–69.7%) overall and 45.4% (95% CI, 31.0–58.6%) for pts with MOD; for adults, Day +100 survival was 48.7% (95% CI, 40.5–56.0%) overall and 43.0% (95% CI, 32.5–53.0%) for pts with MOD. Adverse events (AEs) occurred in 75.4% of the total group (80.6% with MOD); 70.5% of pediatric pts (76.7% with MOD); 78.1% of adults (83.0% with MOD). Treatment-related AEs (TRAEs) occurred in 20.8% overall (23.7% in those with MOD); 21.1% of pediatric pts (23.5% with MOD); 20.7% of adults (23.9% with MOD). The most common TRAEs (>3%) were epistaxis, pulmonary hemorrhage, gastrointestinal hemorrhage, and hemat尿a (each in <5%) of pts. TRAEs leading to study discontinuation (n=25) or death (n=10), the most common was pulmonary hemorrhage.

**Summary/Conclusions:** We conclude that allo-HCT with treosulfan-based conditioning is effective and well tolerated curative therapy for PNH.
P743
HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANT IN SEVERE THALASSEMIA PATIENTS
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Background: Thalassemia free survival after allogeneic stem cell transplantation (SCT) is about 80–90% with either matched related or unrelated donors. However, the probability of finding a HLA-compatible donor is less than 50%. We explored the use of a mismatched related (“Haplo”) donor.

Aims: To evaluate the outcome of SCT with Haplo donors in severe thalassemia patients

Methods: All patients received two courses of pre-transplant immunosuppression therapy (PTIS) with fludarabine (Flu) 40mg/m2/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), 1mg/kg on days SCT -12,-11,-10. Flu 35mg/kg on days SCT -7,-6,-5,-4,-3,-2 and IV Busulfan (Bu) 130mg/m2 on days SCT -7,-6,-5,-4 was given followed by T-cell replete peripheral blood progenitor cells (PBPC). GVHD prophylaxis consisted of cyclophosphamide (Cy) 50mg/kg on days SCT -3 and -4 (Post-Cy), and on day SCT +5 tacrolimus or sirolimus was started together with a short course of mycophenolate mofetil.

Results: Fifty-one patients underwent haplo-SCT. Their median age was ten years (range, 2 to 28 years). Forty-nine patients engrafted with 100% donor chimerism. Two of five patients with high titers of donor-specific anti-HLA antibodies suffered primary graft failure. Median time to neutrophil engraftment was 14 days (range, 11 to 18 days). Eight patients developed mild to moderate, reversible veno-occlusive disease, while twelve patients developed acute GVHD grade II, that quickly responded to steroid therapy. Only seven patients developed limited chronic GVHD. Projected overall and event-free survival rates at two years are 95% and 94%, respectively. The median follow up time is 18 months (range;10 to 50 months).

Summary/Conclusions: This haplo-SCT protocol may yield excellent outcomes for thalassemia patients, and provide a treatment option for patients lacking a HLA-matched donor.

P744
AUGMENTATION OF FLUDARABINE AND BUSULFAN-BASED MYELOABLATIVE REGIMEN WITH THIOTEPA IMPROVES OUTCOMES WITH NO ADDED TOXICITY IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA
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Background: Allogeneic stem-cell transplantation (HSCT, allo-SCT) is the most effective way to control leukemia relapse for patients with acute myeloid leukemia (AML). Busulfan and Cyclophosphamide (Bu/Cy), the current standard of care, in allogeneic transplant for acute myeloid leukemia (AML), is limited by increased treatment related mortality. Myeloablative doses of Busulfan (12-8mg/kg) with Fludarabine (180mg/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 regime 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) in both groups. 5-year disease free survival (DFS) median follow up of 5 years, was significantly better in group 2, 38% for group 1, and 62% in group 2, (p=0.02) and 5-year overall survival showed trend towards benefit in group 2 (62% vs 42%, p=0.06). 14/30 (46%) patients in group 1 relapsed, as compared to 4/31 patients, (12%, p=0.005) in group 2, considering NRM as competing risk.

Summary/Conclusions: In conclusion, the outcome of augmented regimen (DFS and OS) is superior Flu-Bu regime, mainly due to reduction in relapses, with comparable toxicities and could eventually replace Bu/Cy.

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) outcomes 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 associations 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,
in intervals of 5% +/- 2% (e.g. 65 +/- 1.2% probability of survival at 1 year). Corresponding observed 1 year OS was then estimated for each group by the KM method. A kernel smoother was used to visually display the average of observed 1 year survival estimates over the continuous range of predicted OS.

**Results:** 506 patients with AML (n=290), ALL (n=72), or MDS (n=144) were included. Of these, 470 patients (AML=263, MDS=141, ALL=66) had full data available for the CIBMTR Calculator. On univariate and multivariate analyses, DRI, HCT-CI, and age correlated with significant differences in OS/RFS, while donor HLA match correlated with a significant difference in OS. Stratifying patients based on a composite of DRI (low/intermediate vs high/very high) and HCT-CI (0-2 vs 3+) revealed significant differences in OS/RFS between the 4 groups (Fig. 1). Compared with a reference group of patients with both low/intermediate DRI and low HCT-CI, those with high DRI and low HCT-CI were at greater risk of death (HR 2.30; 95% CI 1.39-3.81) and relapse or death (HR 2.50; 95% CI 1.55-4.05), more so than patients with a higher HCT-CI but still low/intermediate DRI (HR death 1.80; 95% CI 1.34-2.43; HR relapse/death 1.68; 95% CI 1.26-2.24). When comparing predicted and observed survival, KM estimates of 1 year OS fell within range of that predicted by the CIBMTR Calculator in almost all groups (Fig. 1). In one group, patients had lower observed 1 year OS than predicted (76%, 95% CI 62-93%, vs 85 +/- 2%, p:NS). In this group, 29/30 patients (97%) had intermediate or high DRI; 59% had poor prognostic ALL by NCCN criteria (n=12, 44%) or other adverse features such as minimal residual disease pre-HCT (n=4, 15%).

**Summary/Conclusions:** Based on a large cohort of patients who underwent CD34 alloHCT for acute leukemia or MDS, we demonstrate that DRI is a major determinant of outcome. The CIBMTR Survival Outcomes Calculator predicts 1 year prognosis with relative precision, though some disease-risk features not reflected in the Calculator may affect outcomes in patients with otherwise good prognosis. Taken together, these prognostic models can assist in predicting outcomes and identifying patients most likely to benefit from CD34 allo-HCT. Furthermore, applying the CIBMTR calculator analysis in individual centers may help identify patients with worse outcomes than predicted and guide patient and/or HCT selection.

**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 common used 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 versus host disease (GVHD). Recently, Blood and Marrow Transplant Clinical Trials Network has proposed a composite endpoint: GVHD-free, relapse-free survival (GRFS) for HSCT outcomes. This endpoint includes as event: 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).

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Aims: We had generated two composite endpoints: in both III-IV aGVHD, relapse or death were considered events but we defined GRFS1 as the one with cGVHD event including those whom required systemic treatment (as the original one) and in GRFS2 just those with severe cGVHD (the EBMT redefined one) and we had compared both.

**Methods:** We retrospectively analysed 616 patients transplanted (1995-2016) excluding non-malignant diseases, second allo-SCT and those <16 years old age.
Efficacy and Safety of Defibrotide in the Treatment of Hepatic Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome Following Hematopoietic Stem Cell Transplantation: Final Subgroup Results


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 (pulmonary dysfunction). The study was amended to also include patients without MOD (off-label), with VOD/SOS per modified Seattle criteria, and/or with VOD/SOS following chemotherapy without HSCT (off-label). After patients provided informed consent, defibrotide treatment (25mg/kg/d in 4 divided doses of 6.25mg/kg) was recommended ≥21 days.

Results: This analysis of final data is based on 1000 patients enrolled from 2007–2016 who had confirmed VOD/SOS following HSCT and had received ≥1 dose of defibrotide. Of these patients, 512 (51.2%) had MOD. The median age was 14 years (range 0.10–77.0), with 570 patients (57.0%) aged ≤16 years, (28.2%) aged <1–12 months, 57.0% aged 13–23 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 myelogenous leukemia (26.1%), and neuroblastoma (10.5%). Kaplan-Meier estimated Day +100 survival was 58.8% (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 (TRA). TRAEs occurring in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).
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 immobilization is hardly achievable.

Aims: We aimed to establish a long term ex vivo culture system that allows maintenance and expansion of LSC (lin- Sca-1+, c-kit+) cells.

Methods: We adapted a technique described by the L. Carlsson lab and transduced high-purity sorted murine LSKs with Lhx2.2, a LIM-homeobox transcription factor, which has been reported to facilitate ex vivo expansion of immature hematopoietic cells.

Results: Lhx2 expressing hematopoietic progenitor cell (HPCCLR) 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. HPCCLR cells repopulate lethally irradiated mice and re-feed the host’s hematopoietic pool. HPCCLR lines were established from a range of transgenic mice, underlining the overall applicability of this model. Using this system, we established LSC lines that express BCR/ABL2, MLL-AF9, NrasG12D or Ifit3-TD; NrasG12D. These LSCs home to the bone marrow, differentiate into all lineages and drive myeloid leukemia in mice.

Summary conclusions: We created an efficient method of expanding hematopoietic stem/progenitor cells. They are immortalized and can be expanded indefinitely. This tool allows analysis of the molecular mechanisms controlling self-renewal in hematopoietic and LSCs as well as drug screening. Our system may represent a breakthrough in (cancer) stem cell biology and assist in the development of new therapeutic avenues to combat LSCs.

P750

INHIBITING BCL2 AND NK CELLS IMPROVES STEM CELL TRANSPLANT OUTCOMES.

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Background: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is the most effective means of preventing relapse of blood cancers, in particular AML. The curative potential of alloHSCT is largely due to the immune mediated graft-versus-leukemia (GVL) effect, which in turn is dependent on the stable engraftment of donor immunity. The dual challenge of alloHSCT is therefore to prevent relapse of disease (involved in early hematopoietic reconstitution) and GVHD, which can be life-threatening.

Aims: We aimed to establish a long term ex vivo culture system that allows maintaining and expanding LSCs that can be transplanted to re-populate irradiated mice.

Methods: We used a MHC-mismatched mouse model of alloHSCT, where lethally irradiated B6 mice were reconstituted with young (8 weeks old) syngeneic bone marrow cells. Recipients were treated with 100mg/kg ABT-199 or vehicle, before receiving alloHSCT. Mice were monitored for more than 6 months. They preserve LSK markers despite continuous proliferation. HPCCLR lines were established from a range of transgenic mice, underlining the overall applicability of this model. Using this system, we established LSC lines that express BCR/ABL2, MLL-AF9, NrasG12D or Ifit3-TD; NrasG12D. These LSCs home to the bone marrow, differentiate into all lineages and drive myeloid leukemia in mice.

Summary conclusions: We created a robust method of expanding hematopoietic stem/progenitor cells. They are immortalized and can be expanded indefinitely. This tool allows analysis of the molecular mechanisms controlling self-renewal in hematopoietic and LSCs as well as drug screening. Our system may represent a breakthrough in (cancer) stem cell biology and assist in the development of new therapeutic avenues to combat LSCs.

P751

MESENCHYMAL STEM CELL IRRADIATION INTERFERES WITH THE ADIPOGENIC/OSTEOGENIC DIFFERENTIATION BALANCE IMPROVING THEIR HEMATOPOIETIC-SUPPORTING ABILITY

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Background: Mesenchymal stem 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 at host-origin. Total body irradiation has been widely used in conditioning regimen and MSC are shown to be radio-resistant. Nevertheless, the functional effects of irradiation on BM-MSC have not been extensively explored.

Aims: The main objective was to evaluate the effects of irradiation on the MSC in their hematopoietic-supporting capacity.

Methods: Ten BM samples were obtained from healthy donors after informed consent. MSC were obtained and characterized following standard procedures and cytokine profiles. The effect of irradiation was assessed by co-culture with T cells. 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 control. MSC were characterized following ISCT criteria (flow cytometry and in vitro differentiation stainings). Apoptosis was evaluated by flow cytometry using annexinV/7AAD staining. Expression microarrays of irradiated and control-MSC were performed using Human Gene 2.0 ST Array platform (Affymetrix). RT-PCR of key genes involved in the hematopoietic supporting capacity as well as in the differentiation of MSC into osteoblasts and adipocytes was performed in both experimental groups. Finally, long term BM cultures (LT-BMC) were performed as functional assays to test the hematopoietic-supporting ability of irradiated and non-irradiated BM-MSC. For the latter experiments, CD34+ cells were isolated from leukapheresis 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 of irradiated MSC with 2,5 Gy improves their hematopoietic supporting ability and modifies their differentiation capacity, increasing the osteogenesis and decreasing the adipogenesis.

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 CAN BE IMPROVED BY N-ACETYL-L-CYSTEINE

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Background: Thrombocytopenia (T) is a common complication in patients with chronic hematologic malignancies, but the underlying pathogenesis is not fully understood. Stem cell transplantation (SCT) is a successful treatment for severe autoimmune disease and bone marrow failure with stem cell transplantation. However, severe T is not improved during stem cell transplantation. In this study, we evaluated whether NAC could ameliorate the clinical symptoms in patients with severe T.

Aims: To evaluate the effect of NAC on bone marrow mesenchymal stem cells (MSCs) in severe T patients.

Methods: The T patients with prolonged isolated thrombocytopenia were enrolled. Bone marrow mesenchymal stem cells (MSCs) from severe T patients were co-cultured with T cells, and then stimulated by irradiation and NAC. Hematopoietic-supporting ability of MSCs was assessed by co-culture with CD34+ cells and CFU-GM colonies were scored weekly.

Results: CFU-GM counts showed the reduction in severe T patients. Compared to control MSC, the CFU-GM counts of NAC MSC were significantly higher (p<0.05). Both NAC and control MSC can reconstitute CD34+ cells, but only NAC MSC can reconstitute the CFU-GM colonies (p<0.05). The apoptosis rate of CD34+ cells showed significant decrease in NAC MSC group (p<0.05).

Summary/Conclusions: NAC can improve the hematopoietic-supporting ability of bone marrow mesenchymal stem cells in patients with prolonged isolated thrombocytopenia. This result suggests that NAC has the potential to be used in the treatment of severe T.

Funding: 81170059, 11575050.

Summary/Conclusions: The findings of this study not only shed light on the pathogenesis of severe T but also provide a potential therapeutic strategy for the treatment of severe T.
Background: Prolonged isolated thrombocytopenia (PT), is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and defined as the engraftment of all peripheral blood cell lines other than platelets (PLT) count ≤20×10^9/L or dependence on PLT transfusions for more than 60 days after allo-HSCT. Several clinical risk factors have been proposed to be associated with PT after allo-HSCT. However, the underlying mechanisms remain to be elucidated. Emerging evidence from mouse studies has suggested that effective hematopoiesis depends on a particular bone marrow (BM) microenvironment in which hematopoietic stem cells reside. MSCs represent a key cellular component of the BM microenvironment, which are potential progenitors for osteoblasts, adipocytes, chondrocytes, and marrow stromal cells. The processes of megakaryocytopoiesis and thrombocytopoiesis result from the interactions between hematopoietic progenitor cells, cytokines, and marrow stromal cells derived from MSCs or MfSCs directly. However, the functional role of BM MSCs in the patients with PT has never been reported. Moreover, approaches for improving the dysfunction of BM MSCs in patients with PT are lacking.

Aims: To evaluated the number and function of BM MSCs derived from patients with PT and its underlying molecular mechanisms in vitro.

Methods: Three cohorts were included: patients with PT (N=25), patients with good graft function (GGF, N=12), defined as persistent successful engraftment after allotransplant, and transplant donors as normal controls (N=10). BM MSCs were cultured as previous reported. All experiments were carried out using BM MSCs derived from passages 2–3. The number and functions of BM MSCs were evaluated by fibroblasts colony-forming unit (CFU-F) assay, cell proliferation assay, cell cycle analysis, and reactive oxygen species (ROS) levels were measured by flow cytometry. Protein expression for p-p38, p38, p-p53, p53 was measured by flow cytometry and western blots. To further investigate the potential effect for repairing the dysfunctional BM MSCs, N-Acetyl-L-cystine (NAC, a ROS scavenger) and SB203580 (p38 inhibitor) were administrated to the BM MSCs for PT patients. After 2 days in vitro culture, the number of SAβ-positive cells was counted, the intracellular levels of ROS and p-p38 were evaluated in BM MSCs by flow cytometry.

Results: Human BM MSCs were demonstrated as spindle shape and typical immunophenotype of MSCs at day 21 of cultivation among subjects with PT, GGF, and normal controls. Cultures from all normal BM samples produced confluent layers of adherent cells composed of spindled shaped cells. 2 of the 12 GGF BM and 15 of the 25 PT BM failed to produce any adherent layers within 3 weeks of culture. BM MSCs derived from PT patients expanded more slowly and appeared flattened and larger. Proliferative capacity and CFU-F counts of BM MSCs from PT patients were significantly reduced compared to those of GGF patients and normal controls. Moreover, increased levels of ROS, which was associated with increased number of SAβ-positive cells, were identified in BM MSCs from PT patients. Intracellular p-p38 level was significantly elevated in BM MSCs from PT patients compared to those in GGF patients. After NAC treatment in vitro, the number of SAβ-positive cells was significantly reduced, whereas the number of senescent cells, the intracellular levels of ROS and p-p38 were reduced markedly in BM MSCs from PT patients.

Summary/Conclusions: In summary, the current study demonstrated the number and the function of BM MSCs were abnormal in PT patients following allo-HSCT. The abnormality was more evident in vitro culture and reversed the senescence phenotype through down-regulation of the p38 MAPK pathway. Our results indicate that the dysfunctional BM MSCs may play an important role in the pathogenesis of PT following allo-HSCT and NAC represents a promising therapeutic approach for repairing the impaired BM MSCs in PT patients post-allograft.

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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 in two different MHC-mismatched bone marrow transplantation (BMT) models and secondly analyzed, whether transplantation of in vitro-generated Th9 cells mediates GVHD.

Methods: We transplanted allogeneic BM and spleen cells from B6-SJL mice (CD45.1, H-2b) in B6D2F1 mice (CD45.2, H-2bd) or in B6.1292 mice (CD45.2, target mice). T cells from Th9 cell-deficient mice (NOD.Cg-Tg(Mch2-I-Abm12) differing either in 50% of MHC class I and II molecules or only in one MHC class II molecule and analyzed the induction of Th9 cell during GVHD development. To clarify whether in vitro-generated Th9 cells mediates GVHD, we induced Th9 cells in vitro from isolated, naïve CD4+ T cells on anti-CD3/CD28 coated plates by Tgf-b, IL-4, anti-Ifng and recombinant Tl1a and co-injected the Th9 cells together with BM in irradiated recipient mice and subsequently monitored GVHD induction.

Results: In both MHC mismatched models used, the transplantation of allogeneic spleen cells and BM leads to GVHD characterized by a time-dependent strong increase of Th1-specific cytokines TNF-a and IFN-g in the serum of the recipients. Surprisingly, IL-9, however, was not induced in the sera of the animals. In both models, allogeneic Th9 cells were identified in the spleen, liver and lung of GVHD-developing animals until 29 days after transplantation, while TNF-a and IFN-g producing cells were strongly increased indicating that Th9 cells are not induced
during GVHD. After in vitro differentiation of Th9 cells from naïve T cells we obtained more than 60% of IL-9 producing cells after 5 days of culture. Th9 cells differ in their cytokine profile (IL-9+, IFN-γ-, IL-13-) from Th1 and Th2 cells. Transplantation of in vitro-generated Th9 cells together with allogeneic BM cells did not induce GVHD in the MHC-disparate recipient mice, while the transplantation of unselected T cells or in vitro-generated Th1 cells induced GVHD and resulted in a median survival of less than 10 days. 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 production and acquired a Th1-like cytokine profile (IFN-γ+, TNF-α, IL-12) pointing to an epigenetic memory of Th9 cells after adoptive transfer. Systemic increase of TNF-α and IFN-γ in the serum of mice receiving Th9 cells, however, was not detected.

Summary/Conclusions: Th9 cells are not induced during GVHD development and the adoptive transfer of in vitro-generated Th9 cells does not induce GVHD. However, the transplanted Th9 cells home to spleen and GVHD target organs and start to produce TNF-α and IFN-γ without strong systemic increase in these cytokines. Since TNF-α and IFN-γ are cytokines associated with an anti-tumor cytotoxicity and Th9 cells are known to eliminate solid tumors, future experiments will define whether in vitro-generated Th9 cells can be used as a cellular therapy for anti-tumor responses in BM-transplanted hosts.

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 transplants. In addition, we have demonstrated that the co-infusion of MSCs with low numbers of purified LSK cells significantly improved the short- and long-term hematopoietic reconstitution in an autologous HSCT experimental model with sublethal conditioning (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 low numbers of MSCs were co-infused. Moreover, when 1,500-3,000 Fanca−/− LSK cells transduced with a therapeutic lentiviral vector (PGK-FANCA-wPRE) were transplanted, the infusion of similar cell doses resulted in more than 50% of engraftment failure, which decreased to 30% only when more than 10,000 gene-corretected LSK were infused. Once again, Ad-MSCs co-infusion prevented graft failure in after 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.

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EFFECT OF POMALIDOMIDE ON T CELL POLARIZATION IS MEDIATED THROUGH EPIGENETIC MODIFICATIONS

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2Children’s Research Hospital, Memphis, United States

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 IMIDs have the potential to modulate naïve T cells towards a Th1 phenotype increasing IFN-γ 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 on the use of pomalidomide as GVHD treatment. Aim: In the current study we have analyzed the effect of pomalidomide in the polarization of CD45RA+ T cells and the epigenetic mechanisms that might be involved in this effect.

Methods: Isolated CD45RA+ T cells from healthy donor’s Buffy Coats were stimulated with anti-CD3 plus anti-CD28 in the presence of several cytokines to polarize towards Th1 (IL-12, INF-γ, anti-IL4) or Th2 (IL-4, IL-2, IL-10, TNF-α 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-IFNγ, anti-CD4 and anti-IL-4 for Th1 cell polarization and anti-CD25, anti-IL-10, anti-CD3 and anti-IL4 for Th2 cell polarization. In addition, the release of cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ) in cell culture supernatants were measured by ELISA. 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 TBET and GATA-3 gene promoters.

Results: Pomalidomide increased the expression of IFN-γ and IL-2 as determined by flow cytometry in Th1 cell culture conditions. By contrast, in the presence of Th2 promoting cytokines we observed an increase for both IL-10 and IL-4 upon adding pomalidomide to the culture. In addition, the exposure to pomalidomide increased the levels of TNF-α, INF-γ 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-γ 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 TBET 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 other gene promoters.

Summary/Conclusions: Pomalidomide favours both Th1 and Th2 cell differentiation 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.

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MESENCHYMAL STEM CELLS (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 (GVHD) 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 development of GVHD. M210B4 cells were administered after allo-HSCT at a dose standardized to mouse/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 phospthase 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 skin fat pad and mesenchyme. MSCs to suppress fibroblast proliferation.

Summary/Conclusions: Our findings indicate that MSCs attenuate the cutaneous manifestation of T cell-mediated immune cell migration and downregulating chemokines and chemokine receptors.
Although C57BL/6N (N) and C57BL/6J (J) mice are derived from the same parental C57BL/6 strain, there are key genotypic and phenotypic differences between these sub-strains. However, more than 58% of studies published involving C57BL/6 mice do not indicate the specific sub-strain employed. J mice have a five-exon deletion in the Nicotinamide nucleotide transhydrogenase (Nnt) gene that results in a non-functional protein. NNT is involved in the resolution of oxidative stress in the mitochondria. Hematopoietic stem cells (HSCs) can reconstitute the entire hematopoietic system after transplantation into hosts whose hematopoietic compartment has been ablated. This is clinically exploited as HSCs transplantation (HSCT) to treat hematologic diseases and represents the only curative therapy for many disorders. During HSCT, HSC are subject to dramatic increases in both intra and extracellular reactive oxygen species (ROS), 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 cells (HSPC) function, here we thoroughly interrogated the frequency and function of HSPCs in J and N bone marrow (BM).

**Methods:** N and J peripheral blood (PB) and BM (n=9) was interrogated by flow cytometry for the absolute frequencies of all major hematopoietic lineages and HSPC compartments, respectively. 5000 J or N CD45.2 HSPCs (Lin-Sca-1+ c-Kit+ cells) were transplanted along with 5000 competitor CD45.1 HSPCs into lethally irradiated mice to test for competitive in vivo hematopoietic repopulating activity and ROS levels post-transplant. The lineage potential and repopulating activity of multi-potent progenitors (MPP2: Lin-Sca-1+c-Kit+Flt3+CD48+CD150-, MPP3: Lin-Sca-1+c-Kit+Flt3+CD48+CD150-, MPF4: Lin-Sca-1+c-Kit+Flt3+CD48+CD150-) was also tested by transplanting 2000 MPPs from J or N mice into sub-lethally irradiated mice and examining the PB of recipients every 3-4 days for 34 days post-transplant. Sensitivity of HSPCs to oxidative stress was tested by examining ROS levels and the in 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 HSPC compartments at 20 weeks post-transplant. However, donor-derived MPPs and CLPs displayed a two-fold increase in ROS levels in recipients of J-HSPCs versus N-HSPCs at 20 weeks post-transplant. MPPs are responsible for repopulation of the hematopoietic system during this early window post-transplant. Different MPP subpopulations can be defined (MPP2, MPP3 and MPP4) according to their self-renewal potential and specific lineage potential. MPP3s and MPP4s are the first MPP subpopulations to reconstitute the lymphoid lineages after transplant. J-MPP3s and J-MPP4s displayed less ROS levels in vivo hematopoietic repopulation activity than N-MPP3s and N-MPP4s. It is known that pI:pC treatment increases ROS levels in HSPCs. We found about two-fold higher ROS levels in HSPCs isolated from pI:pC treated mice than N mice with the exception of J-MPP3s and N-MPP4s. As elevated oxidative stress compromises hematopoietic stem and progenitor cells evaluated the capacity (TIBC), saturation index (SI), serum ferritin (FT) and serum transferrin receptor (TFR).

**Results:** We identified 12 SNPs showing association with the 5 erythrocyte phenotypes previously related to VTE (Table 1). Interestingly, the rs56306145 that showed association with TFR is an intronic variant located in the gene tissue factor pathway inhibitor 2 (TFPI2), which encodes a protein that inhibits a variety of serine proteases of blood coagulation, such as activated factor VII (FVIIa/TF), FXa, plasmin and plasma kallikrein. These data reinforce our previous report of genetic correlation of TFR with VTE. The most significant SNP associations were reported.

**Table 1. Top SNP-associations with erythrocyte phenotypes related to VTE from GWAS in GAIT2.**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>SNP</th>
<th>Chr</th>
<th>Type</th>
<th>Closest gene</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI</td>
<td>rs640</td>
<td>22</td>
<td>Intron</td>
<td>PRKAS</td>
<td>1.00E-10</td>
</tr>
<tr>
<td>RDW</td>
<td>rs20104184</td>
<td>11</td>
<td>Intron</td>
<td>ADH1C</td>
<td>2.10E-01</td>
</tr>
<tr>
<td>BF</td>
<td>rs6502396</td>
<td>5</td>
<td>Intergenic</td>
<td></td>
<td>2.10E-01</td>
</tr>
<tr>
<td>SAT</td>
<td>rs999</td>
<td>7</td>
<td>Intergenic</td>
<td></td>
<td>2.10E-01</td>
</tr>
<tr>
<td>TFR</td>
<td>rs2915415</td>
<td>7</td>
<td>Intergenic</td>
<td>GNOT1</td>
<td>3.10E-01</td>
</tr>
</tbody>
</table>

G: genetic 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.


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**P760**

**ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) PATIENTS SHOW AN INCREASED THROMBUS FORMATION IN A DYNAMIC MODEL OF PLATELET ADHESION**

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Results: Of all patients with major GI bleeding, 14 were taking apixaban (0.8% of all pt on apixaban), 3 (0.4% of all pt on rivaroxaban) rivaroxaban and 1 (0.3% of all pt on dabigatran) on dabigatran. The numbers were too small to identify any statistical difference between the 3 different DOAC drugs.

Summary/Conclusions: The risk of major GI bleeding in our cohort of over 2500 patients over 3 years was noted to be significantly lower than trial data. Since this is a retrospective review from patient hospital database there is a risk of reporting bias and under-reporting of bleeding events. A prospective phase IV study to identify bleeding risk in patients on DOAC is required. Majority of patients with major GI bleeding had other risk factors such as concurrent use of anti-platelets, peptic ulcer disease, alcohol abuse, oesophageal varices, diverticular disease, and bowel malignancy which would increase their bleeding risk on any anticoagulation. Further sub group analysis of this cohort and efforts to improve reporting of anticoagulation associated bleeding is underway.
postoperative day for recognition of DVT/PE was significant greater than 3.5 (7.6 vs 3.5, \( P = 0.048 \)). Patients developing DVT/PE did not differ by obesity or age when compared with non-DVT/PE population.

**Summary/Conclusions:** There have been only a few studies to assess the incidence of DVT/PE in patients undergoing lower limb surgical revascularization. In our study population, 4.4% of patients had evidence of DVT/PE. This presents 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 a pneumatic compression devices (PCDs), over no prophylaxis, to prevent DVT/PE in low risk patients. Since, patients with lower limb surgeries are not a good candidate for PCDs, pharmacological thromboprophylaxis with low dose heparin may be warranted. Given that bleeding is a potential complication in these patients, it might be prudent to start thromboprophylaxis 3.5-4 days after the surgery. Further studies are needed to address 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>( b^2 )</th>
<th>( p (b^2) )</th>
<th>( e^2 )</th>
<th>Covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12 (nmol/L)</td>
<td>441±245 (74-4558)</td>
<td>0.47</td>
<td>2.95 x 10^{-7}</td>
<td>0.11</td>
<td>Age, comorbidities, smoking</td>
</tr>
<tr>
<td>SF (nmol/L)</td>
<td>2.1±4.6 (0.2-41.4)</td>
<td>0.27</td>
<td>2.3 x 10^{-6}</td>
<td>0.07</td>
<td>Sex, comorbidities, smoking</td>
</tr>
<tr>
<td>RCF (nmol/L)</td>
<td>1241±891 (425-3554)</td>
<td>0.42</td>
<td>1.35 x 10^{-5}</td>
<td>0.06</td>
<td>Sex, comorbidities, smoking</td>
</tr>
<tr>
<td>Hcy (nmol/L)</td>
<td>10±4.5 (2.7-97.9)</td>
<td>0.36</td>
<td>3.61 x 10^{-4}</td>
<td>0.41</td>
<td>Sex, comorbidities, 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 m</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>19</td>
<td>ANG</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td></td>
<td>1.3 x 10^{-6}</td>
</tr>
<tr>
<td>RCF</td>
<td></td>
<td></td>
<td>1.2 x 10^{-6}</td>
</tr>
<tr>
<td>HCY</td>
<td></td>
<td></td>
<td>1.2 x 10^{-6}</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** In the GAIT2 study, genetic and environmental factors were related to B12, SF, RCF and HCY. Moreover, a relationship was observed between B12 and VTE. In the GWAS analysis some signals were previously reported (FUT2 and B12 or MTHFR with SF and HCY). New signals were found that need to be clarified, especially their possible relationship with susceptibility to thrombosis.

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**P766**

**CELLULAR ORIGIN OF CIRCULATING MICROPARTICLES (MP) ACCORDING TO SOMATIC MUTATIONS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPN)**

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**Background:** Essential thrombocythemia (ET) and polycythemia vera (PV) are MPN characterized by a high rate of thrombotic complications. We previously demonstrated increased plasma levels of procoagulant MP in ET (Marchetti et al. A.J.H. 2013).

**Aims:** Aim of this study was to extend the analysis of MP to PV patients and to characterize the cellular origin of plasma MP in both ET and PV patients. The influence of somatic mutations [i.e. JAK2V617F, calreticulin (CaiR), thrombopoietin receptor (MPL)], and concomitant cytoreductive or antiplatelet therapies was also evaluated.

**Methods:** Thirty-seven ET (19 JAK2V617F, 9 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 phosphatidylserine on MP.

**Results:** ET and PV patients displayed significantly higher MP levels compared to controls (p<0.05). The majority of circulating MP (90%) were AnnV positive, indicating the expression of phosphatidylserine on their surface. In healthy con-
trols, 71% of MP was positive for platelet (P-MP), 24% for erythrocyte (E-MP), 4% for endothelial cell (EC-MP) and 1% for leukocyte (L-MP) specific markers. In ET and PV patients, the percentage of P-MP was significantly higher (80%; p<0.05), while E-MP level was significantly lower (15%; p<0.05) than controls. L-MP and EC-MP values were comparable between patients and controls. The absolute counts of P-MP and L-MP were higher in both ET and PV versus controls. Overall, no significant correlations were found between the levels of MP derived from platelet, leukocytes or erythrocytes and the corresponding cell counts. The analysis according to patient mutations, revealed significantly higher levels (p<0.05) of both P-MP and E-MP concentration in patients carrying JAK2V617F mutation as compared to JAK2V617F negative patients. In addition, ET patients positive for CaR mutation displayed lower levels (p<0.05) of P-MP compared to JAK2V617F carriers. No influence of concomitant therapies on MP levels or composition was observed.

Summary/Conclusions: Our data confirm the presence of high levels of circulating MP in MPN, which support the role in the known hypercoagulable state of these patients. The MP cellular origin has a different distribution profile according to the presence of different mutations. Importantly, the lack of correlation found between the total and subtype-specific MP counts with the corresponding cell of origin counts suggests an active stimulation of MP formation.

Project funded by AIRC-IG2013 N.14505 of the Italian Association for Cancer Research (AIRC).
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 (IBR) is an oral BTK inhibitor with high response rates in CLL. Venetoclax (VEN) is a potent, highly selective, orally bioavailable small-molecule BCL2 inhibitor. Both IBR and VEN are approved by the FDA and EMA as single agents for chronic lymphocytic leukaemia (CLL). IBR leads to a rapid nodal response with re-distribution of CLL into the peripheral blood whereas VEN leads to depletion of CLL cells to levels in some patients where they cannot be detected. Two of the key cellular processes that are abnormal in CLL are proliferation and apoptosis. The combination of IBR with VEN is therefore logical as biologically the two drugs would be expected to be synergistic. The eradication of minimal residual disease (MRD) from blood and bone marrow is associated with improved outcome in any treatment of CLL where it has been reported. Aims: The CLARITY trial (ISCRTN: 13751862) is a feasibility study to investi- gate the combination and efficacy of IBR combined with VEN in patients with relapsed/refractory CLL. Here we report for the first time the safety of the combination as well as early signs of potent synergy.

Methods: After 8 weeks of IBR monotherapy (420mg/day), VEN was added at a dose of 10mg/day with weekly escalations to 20mg, 50mg, 100mg, 200mg to a final dose of 400mg/day. After the initial 3 patients when there was no sign of tumour lysis syndrome (TLS) the starting dose of VEN was amended to 20mg/day. The primary end-point of the trial is MRD eradication (defined as less than 1 CLL cell in 10,000) in the bone marrow after 12 months of IBR+VEN. Key secondary endpoints are MRD eradication from the bone marrow after 6 and 24 months of combined IBR and VEN as well as the safety of the combi- nation. Important safety events that were considered critical were the incidence of laboratory and clinical TLS. All patients were given prophylactic treatment with uric acid reducing agents beginning at least 72 hours prior to their initial dose of VEN. Over the first three months of combined therapy the level of CLL in the peripheral blood was monitored weekly during VEN escalation and then monthly thereafter. 50 participants will be treated in total.

Results: A total of 35 patients have been recruited between May 2016 and January 2017. To date 21 patients have completed the dose escalation period of VEN with IBR combination with IBR. To date there has been only a single case of labo- ratory TLS in a patient whose phosphate (1.21 to 1.48mmol/l) and creatinine (75 to 146 umol/l) both increased when VEN was increased from 100mg to 200mg. Dosing of VEN was interrupted for 7 days (due to the logistics of clinic closure periods over the Christmas break) and IBR for 24 hours. The biochemical changes were resolved with IBR resuming on the second day and the biochemical TLS was resolved on 400mg/day of VEN with no further TLS. As yet there have been a total of 5 SAES and 22 AE’s of special interest with notably lung infection (n=3) and neu- tropenia (n=11) occurring on more than one occasion. All SAES’s resolved with appropriate management and all AE’s were managed with dose reductions. No SUSAR’s have been reported and no AE’s have been fatal. The level of CLL in the peripheral blood increased during the 8 weeks of IBR monotherapy at 420mg/day from a median of 50 x 10^9/l to a median of 101 x 10^9/l. The rate of fall is rapid in all patients with a median of 0.017 x 10^9/l (range: 0 to 3.1) and then fell from the first 8 weeks of combined IBR with VEN (4 weeks dose escalation fol- lowed by 4 weeks at 400mg/day) from a median of 55 x 10^9/l to a median of 0.017 x 10^9/l (range: 0 to 3.1). The rate of fall is rapid in all patients with a median of 3 log reduction in CLL level after 8 weeks of combined therapy.

Summary/Conclusions: The combination of IBR with VEN is well tolerated in relapsed, refractory CLL with to date only a single case of laboratory TLS. The rapid decline in the level of CLL over the initial 8 weeks of combined therapy phase of VEN with IBR is promising and suggests a potent synergy between the drugs. The initial bone marrow responses are expected after 6 months of combination therapy.

VENETOCXAL IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA WITH 17P DELETION: OUTCOME AND MINIMAL RESIDUAL DISEASE FROM THE FULL POPULATION OF THE PIVOTAL M13-982 TRIAL
1University of Ulm, Ulm, Germany, 2AbbVie Inc, North Chicago, IL, United
CHEMO-FREE TRIPLET COMBINATION OF TGR-1202, UBLITUXIMAB, and IDRUBITIN IS WELL TOLERATED AND HIGHLY ACTIVE IN PATIENTS WITH ADVANCED CLL AND NHL


Methods: Eligible pts had CLL or rel/ref NHL w/o prior to limited therapies, including those ref to PI3Kδ /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), idrubitin dosed at 420mg (CLL) or 560mg (NHL), both on C1D1.

Results: 38 pts treated 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 idrubitin (1 ref/1 rel). MTD was not reached. Most common (>20%) all causality AE’s were fatigue (42%), diarrhea (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 of 34% (all causality) were minimal, the only event >10% was neutropenia (16%). ORR amongst 36 evaluable pts is shown in the following Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>N</th>
<th>CR</th>
<th>PR</th>
<th>ORR</th>
</tr>
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<tbody>
<tr>
<td>CLL/SLL</td>
<td>19</td>
<td>3</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>FL/MZL</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>83%</td>
</tr>
<tr>
<td>DLBCL</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>17%</td>
</tr>
<tr>
<td>MCL</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
</tbody>
</table>

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 analysis of the pivotal M13-982 trial (n=107). Subsequently, 51 additional pts were enrolled in a safety expansion cohort. Aim: To present results from the full trial, including minimal residual disease (MRD) status by both flow cytometry and next generation sequencing (NGS).

Methods: Pts received venetoclax 400 mg daily after initial standard ramp-up until PD or discontinuation due to other reasons. CT scan was mandatory at week 36, after which disease assessment was by clinical evaluation. MRD 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 10 June 2016.

Results: Pts (N=158) had a median age of 67 (range, 29–85) years; a median of 4 (range, 0–10) 32% were fludarabine refractory; 11% had previously received a B-cell receptor signaling inhibitor (BCR); 48% had nodes ≥5 cm; and 78% had unmutated IGHV. The median duration of venetoclax therapy was 16.7 (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 (5.2%), and other (11%). 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 included 5 pts with previously untreated del17p CLL. These pts had an ORR of 80%, CR rate of 40%. All 5 were alive and progression-free at 1 year after their first clinical assessment and 1 pt was positive by flow cytometry (0.008% blood; 9 pts were also negative in the marrow although not necessarily at the same time).

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 inhibitor. MRD negativity by either flow cytometry or NGS correlated with outstanding outcomes.
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 FL). 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 1 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 therapy. PRs have been seen in all 3 cohorts including 10 of 13 (77%) CLL/SLL to peripheral blood in CLL/SLL is consistently observed following one week of therapy. Evidence for tumor cell mobilization to peripheral blood is observed. Of 609 pts with a baseline PET scan, 595 had detectable lesions, and 535 also had an evaluable PET at EOI. Baseline demographics and disease characteristics were similar in PET and non-PET populations. Pts with NA (n=52) or NE (n=8) scans were considered non-responders; these pts and those with PD prior to S1 were excluded from landmark PFS analyses. At EOI, 390/535 (65.5%) pts achieved a PET-CR according to IRC, comprising 212/297 (71.4%) G-chemo pts and 178/298 (59.7%) R-chemo pts. However, for these 390 pts, 50 (8.4%) pts achieved a PET-CR in 161/365 (44.1%) CT-CR and 54 (14%) pts achieved a PET-PR in only 117/362 (32.3%) of pts with CT-PR. Concordance between CT and PET assessment was 52.6% for IRC and 54.1% for INV. Concordance between IRC and INV evaluation was 71.9% for CT and 68.6% for PET. After a median follow-up of 34.5 mo (range 0–54.5), IRC-PET status was highly predictive of PFS (PET-CR vs PET non-CR: HR 0.39; 95% CI 0.25–0.60; p=0.0001) and OS (HR 0.41; 95% CI 0.19–0.86; p=0.018). 2.5-yr PFS from EOI was 87.6% (95% CI 83.5–90.8) for PET-CR pts compared with 70.9% (95% CI 61.3–78.6) for PET non-CR pts; corresponding OS was 96.6% (95% CI 94.1–98.1) vs 90.9% (95% CI 84.7–94.6) (Figure 1).

**Summary/Conclusions:** Cerdulatinib demonstrates clinical activity in heavily pretreated patients with CLL/B-cell NHL and is generally well tolerated. Consistent activity is seen in patients with CLL and FL. Accrual is proceeding; updated PK/PD, safety and efficacy will be presented.

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**Follicular lymphoma • Clinical**

**S774**

**COMPARISON OF CONTRAST-ENHANCED CT-BASED RESPONSE WITH PET RESPONSE AFTER FIRST-LINE THERAPY FOR FOLLICULAR LYMPHOMA IN THE PHASE III GALLIUM STUDY**


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**Background:** Published data show 18F-FDG PET-CT (PET) is predictive after first-line immunochemotherapy in advanced-stage symptomatic FL, and PET is now the recommended modality for response assessment. However, no large-scale prospective comparison of the value of standard contrast-enhanced CT vs PET response has been performed.

**Aims:** To compare CT and PET response assessment for FL pts in the prospective Phase III GALLIUM study, which evaluated chemotherapy plus obinutuzumab (G-chemo) or rituximab (R-chemo) induction followed by maintenance antibody therapy (Marcus 2016).

**Methods:** PET scans, introduced after an early protocol amendment (July 2011), were performed at baseline and end of induction (EOI; all pts gave informed consent) and assessed by the investigator (INV) and an independent review committee (IRC) comprising two radiologists, with a third adjudicator; final response was determined by a clinician. Response was assessed by CT and PET plus bone marrow biopsy, applying the revised International Working Group (IWG) criteria (Cheson 2007, Juweid 2007). Complete remission (CR) status at EOI for each assessment, CT-CR and PET-CR, was compared with pt characteristics, PFS and OS.

**Results:** Among 1202 ITT pts with FL enrolled in GALLIUM, IRC-assessed CT showed a CR in 330 pts (27.5%), PR in 747 (62.1%), SD in 20 (1.7%), PD in 35 (2.9%), unavailable (NA) in 48 (4.0%) and un evaluable (NE) in 22 (1.8%). Of 609 pts with a baseline PET scan, 595 had detectable lesions, and 535 also had an evaluable PET at EOI. Baseline demographics and disease characteristics were similar in PET and non-PET populations. Pts with NA (n=52) or NE (n=6) scans were considered non-responders; these pts and those with PD prior to S1 were excluded from landmark PFS analyses. At EOI, 390/535 (65.5%) pts achieved a PET-CR according to IRC, comprising 212/297 (71.4%) G-chemo pts and 178/298 (59.7%) R-chemo pts. However, for these 390 pts, 50 (8.4%) pts achieved a PET-CR in 161/365 (44.1%) CT-CR and 54 (14%) pts achieved a PET-PR in only 117/362 (32.3%) of pts with CT-PR. Concordance between CT and PET assessment was 52.6% for IRC and 54.1% for INV. Concordance between IRC and INV evaluation was 71.9% for CT and 68.6% for PET. After a median follow-up of 34.5 mo (range 0–54.5), IRC-PET status was highly predictive of PFS (PET-CR vs PET non-CR: HR 0.39; 95% CI 0.25–0.60; p=0.0001) and OS (HR 0.41; 95% CI 0.19–0.86; p=0.018). 2.5-yr PFS from EOI was 87.6% (95% CI 83.5–90.8) for PET-CR pts compared with 70.9% (95% CI 61.3–78.6) for PET non-CR pts; corresponding OS was 96.6% (95% CI 94.1–98.1) vs 90.9% (95% CI 84.7–94.6) (Figure 1).

**Summary/Conclusions:** This large prospective analysis confirms EOI PET as an early predictor of PFS and OS in FL, with good concordance between INV and PET.
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.

**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.

**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**


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**Background:** The Phase III CHRONOS-1 study (NCT01530860) showed that copanlisib (G108-F) significantly prolonged PFS in previously untreated FL pts relative to Gleevec® (G) and was associated with lower serious AEs compared with chemotherapy (CHOP, CVP or bendamustine [B]). Grade 3–5 AEs were more common in G arm compared with B, CHOP or CVP.

**Aims:** To assess outcomes by prior regimens.

**Methods:** Pts with relapsed/refractory FL were randomized 1:1 to G vs B, CHOP or CVP vs G. Pts with PR or CR at EOI (per Cheson 2007) continued to receive G or CHOP vs CVP or B every 2 months for 2 yrs or until progression. The cut-off date for this analysis was October 1 2016. All pts gave informed consent.

**Results:** 241 FL pts were randomized. Baseline characteristics were similar across groups. After 15.3 months’ median follow-up, investigator (INV)-assessed PFS was superior for G (HR, 0.62; 95% CI 0.45–0.85; p=0.003); consistent HRs were observed across groups (Figure 1). HRs for secondary endpoints were supportive. 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 CHOP vs CVP or B.

**Summary/Conclusions:** In relapsed/refractory FL pts, PFS was superior with G relative to chemotherapy with consistent effects across regimens. Some differences were seen in safety profiles between 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.

Aims: We report results from the FL subset of a large phase II study in 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 (40 mg IV infusion) administered on days 1, 8 and 15 of a 28-day cycle until disease progression or unacceptable toxicity. The primary endpoint was objective response rate (ORR) as assessed by independent radiology review according to the response criteria for lymphoma (Cheson et al., JCO 20:579, 2007). Secondary endpoints included progression-free survival (PFS) and duration of response (DOR), safety and tolerability.

Results: A total of 141 patients with iNHL were treated in the phase II study, including 104 patients with FL. The FL subset was characterized as: 52% male, 83% white, median age 62 years, 62% ECOG 0, 63% refractory to last therapy, median time from most recent progression 8 wks (range 1-73) and median prior lines of therapy 3 (range 2-8). At the time of primary analysis the ORR was 58%, comprising 15 patients (14.4%) with complete response 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 wks (range 1-103); 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 (24%/6%), reduced neutrophil count (30%/24%), fatigue (30%/2%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/14%), hepatic enzycympathy (AST 28%/14%; ALT 23%/14%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to copanlisib: one lung infection, one respiratory failure, and one thromboembolic event.

Figure 1.

Summary/Conclusions: Copanlisib was highly active as a single agent in heavily pretreated relapsed/refractory FL patients and resulted in responses in the majority of patients with a median duration of response exceeding one year. Toxicities were manageable, with a low incidence of severe AEs associated with other PI3K inhibitors, especially hepatic enzycympathy, opportunistic infections, and colitis.

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DYNAMO: A PHASE 2 STUDY DEMONSTRATING THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH DOUBLE-REFRACTORY FOLLICULAR LYMPHOMA


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Background: Duvelisib is an oral, dual inhibitor of PI3K-δ,γ in development for the treatment of hematologic malignancies. DYNAMO is a Phase 2 study to evaluate the safety and efficacy of duvelisib monotherapy in a double refractory iNHL population, which included a majority of patients (pts) with follicular lymphoma (FL).

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 profile of duvelisib.

Methods: DYNAMO is an open-label, single-arm, safety, and efficacy study in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL), whose disease is double-refractory to rituximab (monotherapy or in combination) and to chemotherapy or radioimmunotherapy. Pts who had received ≥25 mg/m² of duvelisib 21 days prior to the study were excluded. The study enrolled 111 pts with relapsed/refractory FL, in whom duvelisib monotherapy was administered at doses of 150 mg twice daily, with or without PJP prophylaxis. Pts were excluded if they had a history of brain metastasis, any prior treatment with a PI3K inhibitor, or any history of lymphomatous polyposis.

Results: A total of 134 pts with FL were evaluable for efficacy and safety in the primary analysis. Among the 111 FL pts with a response per IRC, the median DoR was 9.2 months. The median PFS for all FL pts was 8.3 months, while the median OS was 25.8 months. Among the 34 FL pts with a response per IRC, the median DoR was 9.2 months. The median TTR was 1.9 mo. (range: 1.6 - 11.7). 80% of FL pts experienced disease progression or unacceptable toxicity. The primary endpoint is overall response rate (ORR) as assessed by an independent review committee (IRC) per revised IWG criteria. Secondary endpoints include duration of response (DoR), progression-free survival (PFS), overall survival (OS), time to response (TTR), adverse events (AEs) and other safety parameters.

Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory FL population (41% ORR, median DoR 9.2 mo., 80% with disease progression or unacceptable toxicity. 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 profile of duvelisib.
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, aalPI 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

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PHASE II TRIAL OF COMBINATION OF ELOTUZUMAB, LENALIDOMIDE, AND DEXAMETHASONE IN HIGH-RISK SMOLDERING MULTIPLE MYELOMA

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Aims: The overarching objective of this trial is to determine progression free survival to symptomatic myeloma (MM). Furthermore, the study examined whether genomic studies can help in determining patients who would benefit the most from early therapeutic intervention.

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 with NDMM (SCRT-eligible) received twice-weekly oral ixazomib (3.0 or 3.7 mg on days 1, 4, 8, and 11) plus lenalidomide (20 mg [10 mg in cycles 9–16] on days 1, 2, 4, 5, 8, 9, 11, and 12) for up to sixteen 21-day cycles, followed by maintenance therapy with single-agent twice-weekly ixazomib. Patients not proceeding to SCT did not receive further ixazomib therapy. Response/progression was assessed per IMWG criteria after cycles 1, 2, 3, 4, and then every 2 cycles during induction and maintenance.

Results: Of the 64 enrolled patients, 40 continued on study treatment without withdrawal for SCT long-term follow-up of these 40 patients is reported here. The median age of patients was 66 years (range 34–82), and 45%/38%/18% of patients had ISS disease stage I/II/III. At a median follow-up of 47.0 months, the overall response rate (ORR; partial response [PR] in the absence of progression) was 94%, the complete response rate (CR+VGPR) rate was 68%, and the CR rate was 32%. Median time to first response was approximately 1 cycle (0.72 months). Median time to a best response of CR was 4.2 months. Patients received a median (range) of 14 (1–75) treatment cycles. Median progression-free survival (PFS) for patients not proceeding to SCT was 24.9 months. Median overall survival (OS) was not estimable; the 2-year Kaplan-Meier estimate for OS was 92%. A total of 78% of patients had grade ≥3 treatment-related adverse events (AEs); the most common treatment-related grade ≥3 AEs and serious AEs are shown in the Table 1. After completing induction therapy with IRd, 18 patients went on to receive maintenance with single-agent ixazomib on a twice-weekly dosing schedule. Patients who went on to maintenance received a median (range) of 31.5 (17–75) treatment cycles. Among the patients who received maintenance therapy, the ORR (PR%) was 54%, the CR+VGPR rate was 67%, and the CR rate was 44%. Two (11%) patients improved their responses during maintenance. Common treatment-related grade ≥3 AEs occurring in ≥25% of patients are shown in the Table 2.

Table 1. Common treatment-related grade ≥3 AEs occurring in ≥25% of patients

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<tr>
<th>AEs</th>
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<tr>
<td>Grade ≥3 treatment-related AEs occurring in ≥25% of patients</td>
<td>Data in %</td>
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<tr>
<td>Nausea</td>
<td>25%</td>
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<td>Neutropenia</td>
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<td>Anemia</td>
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Table 2. AE definitions

<table>
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Aims: This phase II study of elotuzumab, lenalidomide, and 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 2013).

Results: Of the 64 enrolled patients, 40 continued on study treatment without withdrawal for SCT long-term follow-up of these 40 patients is reported here. The median age of patients was 66 years (range 34–82), and 45%/38%/18% of patients had ISS disease stage I/II/III. At a median follow-up of 47.0 months, the overall response rate (ORR; partial response [PR] in the absence of progression) was 94%, the complete response rate (CR+VGPR) rate was 68%, and the CR rate was 32%. Median time to first response was approximately 1 cycle (0.72 months). Median time to a best response of CR was 4.2 months. Patients received a median (range) of 14 (1–75) treatment cycles. Median progression-free survival (PFS) for patients not proceeding to SCT was 24.9 months. Median overall survival (OS) was not estimable; the 2-year Kaplan-Meier estimate for OS was 92%. A total of 78% of patients had grade ≥3 treatment-related adverse events (AEs); the most common treatment-related grade ≥3 AEs and serious AEs are shown in the Table 1. After completing induction therapy with IRd, 18 patients went on to receive maintenance with single-agent ixazomib on a twice-weekly dosing schedule. Patients who went on to maintenance received a median (range) of 31.5 (17–75) treatment cycles. Among the patients who received maintenance therapy, the ORR (PR%) was 54%, the CR+VGPR rate was 67%, and the CR rate was 44%. Two (11%) patients improved their responses during maintenance. Common treatment-related grade ≥3 AEs occurring in ≥25% of patients are shown in the Table 2.

Table 1. Common treatment-related grade ≥3 AEs occurring in ≥25% of patients

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Summary/Conclusions: In patients with NDMM, twice-weekly ixazomib plus Rd resulted in excellent 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.

S782

COMPARISON OF DENOSUMBOT WITH ZOLEDRONIC ACID FOR THE TREATMENT OF BONE DISEASE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; AN INTERNATIONAL, RANDOMIZED, DOUBLE-BLIND TRIAL


1Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece, 2Indiana University Simon Cancer Center, Indianapolis, United States, 3Medical University of Innsbruck, Innsbruck, Austria, 4National Hospital Organization Higashi Nagoya National Hospital, Nagoya, Japan, 5Hospital Universitario de Salamanca, Salamanca, Spain, 6Cedars-Sinai Medical Center, Los Angeles, 7Amgen Inc., Thousand Oaks, 8Massachusetts General Hospital Cancer Center, Boston, United States

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 withZA in newly diagnosed myeloma pts.

Methods: In 2184 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-previous) 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 68% of DMB and 67.2% of ZA pts reporting prior SRE history; CrCl<30mL/min was reported in 26.7% of pts. During the primary blinded treatment period (median follow-up 17.4 months [m]), 43.8% DMB pts and 44.6% ZA pts had a first on-study SRE. The median time to first on-study SRE was similar between DMB (22.83 m) and ZA (23.98 m) pts. DMB was non-inferior to ZA in delaying time to first on-study SRE (HR[95%CI]=0.98[0.85,1.14]). Superiority was not demonstrated for time to first on-study SRE (P=0.82) and time to first-and-subsequent on-study SRE (P=0.84). In this high-risk study population the effect of antiresorptive therapy may only be evident later in the treatment course. A post-hoc, landmark analysis at 15 m for time to first SRE demonstrated a HR[95%CI]=0.66[0.44,0.98], 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 (129[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 46.09m (95%CI:34.3,NE) for DMB and 35.38m (95%CI:30.19,NE) for ZA. The most common AE (≥25%) for DMB and ZA 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 positive adjudication ONJ (4.1,2.8) were comparable to known safety profiles. Fewer DMB pts (%) compared with ZA pts had AEs potentially related to renal function (10.0,17.1;P<0.001), most notably in pts with baseline CrCl≤60mL/min (12.9,11.5).

Figure 1. Summary/Conclusions: DMB demonstrated non-inferiority to ZA in delaying time to first on-study SRE in myeloma pts, meeting the primary endpoint of the study. A landmark analysis at 15 m suggests a significant benefit for DMB with respect to time to first SRE. The rates of renal AEs were significantly lower in DMB pts while the overall rates of AEs, including hypocalcemia and ONJ, were consistent with the known DMB safety profile. The results of the landmark analysis and possible prolongation of PFS with DMB therapy is promising.
Background: Pembrolizumab (pembro) is a humanized, highly selective, high-affinity IgG4/Aκ antibody that blocks the interaction between programmed death 1 (PD-1) and its ligands PD-L1 and PD-L2, activating antitumor 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 pembro 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 (NCT02038502) study of pembro 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 pembro 200 mg IV every 2 weeks (Q2W), len 25 mg orally on days 1-21, and dex 40 mg day 1 (C1D1); and cycle 2, day 1 (C2D1) blood samples.

Results: MTD was determined as pembro 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 AEs (TRAEs) were neutropenia (33%), thrombocytopenia (18%), and anemia (12%). 2 patients (4%) died because of TRAEs (hepatic failure, ischemic stroke). Immune-related AEs occurred in 5 (10%) patients. No pneumonitis was reported. ORR in the efficacy population was 39/40 (98%) in the efficacy population and 37/38 (97%) in the safety population. The disease control rate for len- and double-refractory patients, respectively. The disease control rate (sCR+CR+ VGPR+PR+SD) was 39/40 (98%) in the efficacy population and 29/28 (97%) in the len-refractory population. 35/40 (88%) patients had a reduction in M protein or free light chains. In 16/32 patients with FC-evaluable BM aspirate with >100 CD38+CD138+ cells, all were PD-L1+, while PD-L2 expression was variable. At C2D1, proportion of circulating HLA-DR+, central (CD45RO+CCR7−), and effector memory (CD45RO+CCR7+) CD8+T cells was variable. At C2D1, proportion of circulating HLA-DR+, central (CD45RO+CCR7−), and effector memory (CD45RO+CCR7+) CD8+T cells signif-

Summary/Conclusions: The combination of pembro, len, and low-dose dex induced immune activation in the periphery and a phenotypic shift in effector CD8+ T cells among the circulating T-cell pool in blood.

RUXOLITINIB FOR THE TREATMENT OF INADEQUATELY CONTROLLED POLYCYTHEMIA VERA WITHOUT SPLENOMEGALY: 80-WEEK FOLLOW-UP FROM THE RESPONSE-2 TRIAL

RUXOLITINIB (RUX) is a Janus kinase (JAK) inhibitor approved for the treatment of hydroxyurea (HU)-resistant/intolerant pts with PV ≥18 years of age. The RESPONSE-2 trial (NCT01743603) evaluated the efficacy and safety of RUX in HU-resistant/intolerant pts with PV ≥18 years of age randomized 1:1 to RUX 10 mg twice daily or best available treatment (BAT). Primary end point was the proportion of pts who achieved hematocrit (HCT) ≤45% at wk 80. house (HU)-resistant/intolerant pts with PV ≥18 years old and new drugs in MPN

Old and new drugs in MPN

POLYCYTHEMIA VERA WITHOUT SPLENOMEGALY: 80-WEEK FOLLOW-UP FROM THE RESPONSE-2 TRIAL

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PHASE 3 RANDOMIZED TRIAL OF MOMELOTINIB VERSUS RUXOLITINIB IN JAK INHIBITOR NAIVE PATIENTS WITH MYELOFIBROSIS: RESULTS OF THE SIMPLIFY-1 STUDY

1Stanford University Medical Center, Stanford, United States, 2Saint-Louis Hospital (APHP) and Paris Diderot University, Paris, France, 3Monash University, Melbourne, Australia, 4University Hospitals Leuven, Leuven, Belgium, 5Kaposi Mar Teaching Hospital, Kapovaro, Hungary, 6Medical University of Gdańsk, Gdańsk, Poland, 7Guy’s and St. Thomas’ NHS Foundation Trust, London, United Kingdom, 8University of Miyazaki, Miyazaki, Japan, 9Emory University School of Medicine, Atlanta, 10Gilead Sciences, Inc., Foster City, United States, 11Hospital Clinic, University of Barcelona, Barcelona, Spain, 12Mayo Clinic Cancer Center, Scottsdale, United States

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 requirement, in JAKi naive patients with primary myelofibrosis, and post-polycythemia vera or post-essential thrombocythemia myelofibrosis.

Methods: Eligibility included primary myelofibrosis or post-polycythemia vera/essential thrombocythemia myelofibrosis; International Prognostic Scoring System (IPSS) high risk, intermediate-2 risk, or intermediate-1 risk associated with symptomatic splenomegaly; palpable spleen ≥5cm; platelets ≥50 K/μl; no Grade ≥2 peripheral neuropathy. informed consent was obtained. Stratification was by transfusion dependency 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).

Results: Of 73 of 104 (70%) and 40 of 52 (77%) patients receiving MMB or BAT, respectively, completed the 24 week randomized treatment phase. BAT for patients included ruxolitinib, and 27% of patients were on ruxolitinib in combination with other drugs. Efficacy results are 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.

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Summary/Conclusions: In patients with JAKi naïve myelofibrosis, 24 weeks of momelotinib is non-inferior to ruxolitinib for spleen response but not for symptom response. Momelotinib treatment is associated with a reduced transfusion requirement. NCT01969838

S786

PHASE 3 RANDOMIZED TRIAL OF MOMELOTINIB VERSUS BEST AVAILABLE THERAPY IN PATIENTS WITH MYELOFIBROSIS PREVIOUSLY TREATED WITH RUXOLITINIB: RESULTS OF THE SIMPLIFY-2 STUDY

1University of Texas MD Anderson Cancer Center, Houston, United States, 2University of Florence, Florence, Italy, 3Medizinische Fakultät Carl Gustav Carus, Technische Universität, Dresden, Germany, 4Hospital Clinic, University of Barcelona, Barcelona, Spain, 5Princess Margaret Cancer Centre, University of Toronto, Toronto, Canada, 6Hadassah-Hebrew University Medical Center, Jerusalem, Israel, 7University of Insibria, Varese, Italy, 8Emory University School of Medicine, Atlanta, 9Gilead Sciences, Inc., Foster City, United States, 10Saint-Louis Hospital (APHP) and Paris Diderot University, Paris, France, 11Guy’s and St. Thomas’ NHS Foundation Trust, London, United Kingdom

Background: Momelotinib (MMB), an investigational oral JAK inhibitor, has been shown in early trials to reduce spleen volume, improve disease-associated symptoms and improve red blood cell (RBC) transfusion requirements in patients with myelofibrosis.

Aims: To test the superiority of MMB versus best available therapy (BAT) in splenic volume reduction, symptom amelioration, and transfusion requirement at 24 weeks in patients with primary myelofibrosis (PMF), post-polycythemia vera or post-essential thrombocythemia myelofibrosis (Post-PV/ET MF) who were previously treated with ruxolitinib.

Methods: Eligibility included PMF or post-PV/ET MF; Dynamic International Prognostic Scoring System (DIPSS) high risk or intermediate-2 risk, or intermediate-1 risk associated with symptomatic splenomegaly; previously treated with ruxolitinib for at least 28 days who either required transfusions or dose reduction to <20 mg BID with at least one Grade ≥3 thrombocytopenia, anemia, or bleed; palpable spleen ≥5cm; and no Grade ≥2 peripheral neuropathy. Informed consent was obtained. Stratification was by transfusion dependency and platelets (<100K, 100K-200K, and >200K/μl). Patients were randomized 2:1 to 24 weeks of open-label MMB 200 mg QD or BAT. Assessments included spleen volume by MRI, and patient-reported symptoms using a daily eDiary of modified MPN-SAF Total Symptom Score (TSS). Primary endpoint was spleen response rate at 24 weeks (SRR24; ≥35% reduction in volume from baseline). Secondary endpoints, evaluated sequentially, were rates of TSS response (TSS RR; ≥50% reduction from baseline), RBC transfusion, RBC transfusion independence (TI), and RBC transfusion independence (TD).

Results: Of 320 (70%) and 40 of 52 (77%) patients receiving MMB or BAT, respectively, completed the 24 week randomized treatment phase. BAT for patients included ruxolitinib, and 27% of patients were on ruxolitinib in combination with other drugs. Efficacy results are 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.

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*Test for non-inferiority; °Test for superiority; all 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

J.-J. Kladjan1,2,3,4,5, B. Cassinat2,4, J. Soret-Dulphy6, E. Verger7, L. Roy6, J. Rey8, N. Masliah9, B. Grohmann-İzay10, C. Klaide11, H. Gisslinger11,2,12

1Clinical Investigations Center, Hopital Saint-Louis, 2INSERM UMRs-1131, 3Paris Diderot University, 4Hôpital Saint-Louis, Paris, 5Hôpital Henri Mondor, Créteil, 6University of Caen, Caen, 7NAP orphan Pharmaceuticals AG, 8Medical University of Vienna, Vienna, France

Background: Interferon alfa (IFNa) has been successfully used to treat myeloproliferative neoplasms (MPN) for many years and several phase 2 studies have independently 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: We report the randomized controlled phase III trial (PROUD-PV) comparing the novel, long-acting Rogepinterferon 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 (CHG) according to ELN criteria and normal spleen size. An important secondary endpoint the effect of treatment on %JAK2V617F was assessed as rate of complete and partial molecular response (C/PMR) according to modified ELN criteria. In the group of pts enrolled in France, we could study BM progenitors 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 CHG 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) CHG at 12 mos was 40% in pts receiving AOP2014 (n=5) and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46.6% respectively (p=0.08). HU patients were older (median age 61, 5 of 12 vs. 65, 7 of 12 years, respectively; p=0.03). AOP2014 treatment induced an important decrease of the proportion of EECs in cultures with or without EPO at baseline as well as 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 pts (median decrease 25%) while it was not different in the baseline at 12 months of therapy in AOP2014-treated pts. Among EUC-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 Rogepinterferon alfa-2b versus HU, we found a different impact of both drugs on hematopoietic cells. Although both treatment induced a decrease of JAK2 mutant allele burden at 12 mos in peripheral blood, BM clonogenic assays suggest that AOP2014 is able to specifically target JAK2 mutant progenitors, an effect not seen in HU treated pts. Such targeted impact of AOP2014 may account for the strikingly different kinetics in allele burden reduction and suggests that sustained long-term molecular response may only be achieved with IFNa based treatment.

Table 1.

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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: AdSM (ie, aggressive SM [ASM], SM with an associated hematologic neoplasm [SM-AHN], and mast cell leukemia [MCL]) comprises rare hematologic neoplasms with a poor prognosis. Kit D816V mutations occur in a majority of patients with adSM. 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 cohort of SM. 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 analyses did not include matching for patient selection, subgroup analyses, and multivariate analyses were performed to assess the impact of baseline patient characteristics affected OS and estimated HR. Propensity scoring was used for supportive analyses to match the patients in the registry. Patients were evaluated for OS based on time from diagnosis to death; patients in the pooled analysis with known dates of diagnosis were included in the primary analysis. A sensitivity analysis to compensate for potential bias of patient selection was conducted using the start date of last treatment to death.

Results: The primary analysis of OS in patients with adSM included 89 patients from the midostaurin pooled analysis for whom the date of diagnosis was available (77% of the entire pooled cohort) and all 46 patients from the German registry who had not been treated with midostaurin. SM subtypes among patients from the pooled analysis and registry were similar; 66% of patients in the pooled cohort and 63% in the registry had an AHN (Table 1). Kit D816 mutations were present in 82% of patients in the pooled analysis and 96% in the registry. More patients in the registry (67%) vs the pooled analysis (42%) were aged >65 y. Median follow-up (time from diagnosis to data cutoff for the analyses) was similar for the 2 patient groups: registry, 54.9 (range, 1.9-150.4) mo and midostaurin, 53.6 (range, 31.6-215) mo. Patients in the midostaurin pooled analysis had a clinically relevant improvement in OS vs historical controls (HR=0.62 [95% CI, 0.39-0.98]; P=0.024; Figure 1). Median OS was 42.8% (95% CI, 31.0-53.9) mo in the pooled analysis vs 24.0% (90% CI, 13.0-39.5) mo in the registry. Multivariate Cox regression analysis after adjusting for covariates was consistent with the primary analysis: HR=0.51 (95% CI, 0.30-0.88); P=0.0147. Data using propensity score for matched pairs (n=44) were also consistent (HR=0.38 [95% CI, 0.22-0.65]; P=0.0004).

Table 1.

In this phase 3 trial comparing Rogepinterferon alfa-2b versus HU, we found a significant improvement of immune cell function in the activated state as compared to HU. Although both treatment induced a decrease of JAK2 mutant allele burden at 12 mos in peripheral blood, BM clonogenic assays suggest that AOP2014 is able to specifically target JAK2 mutant progenitors, an effect not seen in HU treated pts. Such targeted impact of AOP2014 may account for the strikingly different kinetics in allele burden reduction and suggests that sustained long-term molecular response may only be achieved with IFNa based treatment.
Midostaurin was associated with a 38% lower risk of death vs historical controls. Benefit was generally consistent across key subgroups.

**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 cytarabin 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 cytarabin treatment (1.5 mg/kg i.v./s.c. daily) for one week respectively if they met the following criteria: TMD-related symptoms (n=59), no significant differences were observed regarding EFS (81±5% vs 71±5%, p=0.15) than patients without these symptoms in the historic control (n=101).

**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 cytarabin 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).

**Summary/Conclusions:** The outcome 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 cytarabin 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 novel 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) in case of leukocyte index (LI) leukocytosis (BM blasts<10%) ≤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 internal tandem duplication (FT3-ITDwt) and no MLL rearrangement: ASCT. Adverse risk (AR), patients not considered in FR or IR or ASCT or allo-SCT or allo-SCT (allo-SCT) depending on donor availability (HLA-identical sibling or unrelated donor if high risk of relapse).

Results: There were enrolled 868 patients. Median age was 53 years-old (16-70). According to MRC cytogenetics, available in 802 patients, 99 belonged to the favorable (12%), 581 (73%) to the intermediate and 122 (15%) to the adverse groups. 66 patients with no metaphases. FLT3-ITD was present in 128 patients with normal karyotype (36%). Four patients died before treatment and 864 patients received induction therapy. 77% of patients achieved a CR (88% with a single course), 11% were refractory and 12% died during induction. CR rates were 94% in patients with normal karyotype and no mutations, 91% in NPM1 mutation without FLT3-ITD, 77% in intermediate cytogenetic and no mutations, 74% if FLT3-ITD, 70% in adverse cytogenetics and 62% if monosomal karyotype was present (p<0.001). The multivariate analysis showed that mutational status (adverse cytogenetics, FLT3-ITD and absence of NPM1 mutation) had an adverse impact on CR achievement. The percentage of CR (OS) -evaluated in patients cured in CR and cell expansion (allo-SCT) during study period, we identified 13 cases (maintenance) and 26 controls using log rank test and cox proportional hazards regression analysis. In this, the largest such study reported to date, the demonstration that mutations in CDKN2A, IDH1 and TP53 are associated with reduced OS and survival, and that FLT3-ITD mutation is a genetic alteration found in approximately 30% of patients with AML who did not receive maintenance post SCT(control group); we identified mutations in CDKN2A (p=0.0001), IDH1 (p=0.004) and TP53 (p=0.003), NPM1 (p=0.037) and FLT3-ITD (p=0.04) as significant molecular markers for survival, and 82% of these patients were associated with reduced OS.

Summary/Conclusions: In this, the largest such study reported to date, the demonstration that mutations in CDKN2A, IDH1 and TP53 are associated with reduced OS and survival, and that FLT3-ITD mutation is a genetic alteration found in approximately 30% of patients with AML who did not receive maintenance post SCT(control group); we identified mutations in CDKN2A (p=0.0001), IDH1 (p=0.004) and TP53 (p=0.003), NPM1 (p=0.037) and FLT3-ITD (p=0.04) as significant molecular markers for survival, and 82% of these patients were associated with reduced OS.

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SORAFENIB MAINTENANCE IN FLT3-ITD MUTATED ACUTE MYELOID LEUKEMIA AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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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 FLT3-ITD mutated AML achieve remission rates similar to those with FLT3 wildtype status with induction chemotherapy regimens; patients with FLT3-ITD have significantly shorter remission durations and increased rates of relapse. Even though allogeneic SCT improves outcomes, patients still have higher rates of relapse compared to patients with FLT3 wildtype status.

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 (aTAC) treated between 1/2010 and 7/2015. FLT3-ITD mutated AML who did not receive maintenance post SCT(control group); we matched each case to two control patients accounting for disease status, type of SCT, donor type and donor status. For patients who received maintenance post SCT, the study was censored at the time of last contact.

Summary/Conclusions: The CIR when comparing ASCT (p=0.033) and allogeneic SCT (p=0.003) as well as identify molecular predictors of outcome in 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.

Background: In the RAVVA study, a randomized II trial which compared AZA monotherapy with AZA/VOR combination therapy. To analyze the results of intensive induction and post-remission treatment in 250 patients treated on the RAVVA trial and correlated with response.
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 (CnR) in 69% while it was active disease in 31%. PFS at 24 months post SCT was 82% in the maintenance and 45% in control group HR 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 SBF 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.

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**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 only) with 7+3. Most patients had blast clearance (CR+CRi+mLFS), 73% (27/37) achieved MRD negative status. The CR 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. Across schedules (N=67), the CR rate was 76%; 79% (44/56) of evaluable patients with blast clearance achieved MRD negativity. The 30- and 60-day mortality rates were 1% and 7%, respectively. Median OS is not reached for either schedule and 52 patients (78%) were alive at the time of analysis.

**Summary/Conclusions:** 33A can be safely combined with 7+3 with acceptable count recovery in this population at the doses and schedules studied. Extramedullary AEs, including hepatotoxicity, and induction mortality rates were similar to reported rates for 7+3 alone in this AML population. A high remission rate with the 1st induction cycle was observed, the majority of which were MRD negative.
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-IV 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, memory T, regulatory (Treg), Th1, Th2, Th17, T follicular helper (Tfh), Th9, Th22, ThGM-CSF cells, granulocytes, monocytes, myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, and monocytes 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 expression of NK92 and NK10 T cells (CD3-CD20-CD14-CD56+, mDCs (CD3-CD20-CD14-HLADR+CD56+), mDCs (CD3-CD20-CD14-HLADR+CD11c+), and memory CD4+ T cells (CD3+CD4+CD45RA-). Among CD4+ memory cells, the CR group showed significant or trend-toward-significant increases in Tfh (CXCR5-CX3CR1+C), Th1 (CXCR5-CXCR6-CX3CR1+CXCR10+), Th2, Th17 (CXCR5-CXCR6+CXCR4+CX3CR3-CXCR10-), Th17 (CXCR5-CXCR6-CX3CR3-CXCR10-), Th22 (CXCR5-CXCR6+CX3CR3-CXCR10-), and Th22 (CXCR5-CXCR6+CX3CR3-CXCR10-). 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+) (84.7% vs 38.0%, CR vs PR/MR, p=0.0078) and away from MDSCs (HLADR+CD11c+) (30.0% vs 58.4%, CR vs PR/MR, p=0.0139) during treatment. Interestingly, the NK-to-MDCS ratio was a sensitive and specific predictor of CR vs all other responses, a finding consistent for both CD16+ and CD16- NK cells (Figure 1 a, b). Before INCB039110 treatment, decreased naive CD8+ T cells (CD45RA+CCR7+) predicted CR versus PR/MR (12.6% vs 32.3% of CD8+ cells, CR vs PR/MR, p=0.0047) with a similar trend toward decreased naive CD4+ T cells (13% vs 24.4% of CD4+ cells, CR vs PR/MR, p=0.0749). While naïve T cells did not correlate with pretreatment aGVHD grade, grades III-IV aGVHD demonstrated increased Th2 cells (CD45RA-CCR5+CCR6-CX3CR3+CXCR10-) and activated CD8+ T 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.

Figure 1.

Summary/Conclusions: Decreased pre-treatment naïve 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-MDCS 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.

GUT COLONIZATION BY MULTI-DRUG RESISTANT BACTERIA IS AN INDEPENDENT RISK FACTOR FOR DEVELOPMENT OF INTESTINAL ACUTE GRAFT-VERSUS-HOST DISEASE

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Background: Research has recently highlighted the importance of healthy gut microbiota in the prevention of graft-versus-host disease (GVHD). Gut decontamination and the use of broad-spectrum antibiotics have led to the loss of natural microbiota diversity and the overgrowth of opportunistic pathogens with emerging antimicrobial resistance. However, the role of multi-drug resistant by VSR is still under development. G. Vrhovac et al.

Aims: Our aim was to evaluate the impact of gut colonization with MDR bacteria on the acute GVHD and related outcome.

Methods: Retrospectively we evaluated 145 adult patients who consecutively underwent allologeneic stem cell transplantation (allo-SCT) in our institution between 2011 and 2014. All patients were weekly screened by cultivating stool specimens for gut colonization by the following MDR bacteria: vancomycin-resistant Enterococcus (VRE), methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Gram-negative bacilli (MDR-GNB). Univariate and multivariable proportional hazards models using the Fine and Gray approach were considered to evaluate the variables for acute GVHD, treating death as competing event.

Results: Our study population included 88 male and 57 female patients who underwent allo-SCT at a median age of 46 years (range 18-64). Among them, most patients were treated for myeloid malignancies (70%) and had lymphoproliferative disorders and one patient had aplastic anemia. The donors were unrelated in 74 cases, related in 67 patients and haploidentical in 4 patients. Most of the patients (70%) received peripheral blood stem cells after a reduced-intensity conditioning regimen (56%). At the time of allo-SCT 37% patients were colonized with MDR bacteria, while another 19% became colonized in the early postransplantation period. Among colonized patients, 12% patients were colonized by VRE, 1% by MRSA, 43% by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, 27% by carbapenem-resistant Enterobacteriaceae (CRE), 9% by MDR Acinetobacter baumannii and 50% by 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 GVHD.

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.
Advanced total body irradiation (TBI)-based chemotherapy-based regimens (Cy/TBI or etoposide (Vep/TBI).

**LEUKEMIA WORKING PARTY OF THE EBMT**

**TOTAL BODY IRRADIATION AS CONDITIONING FOR ADULTS WITH CYCLOPHOSPHAMIDE**

The advantage of TBI-based chemotherapy-based regimens (Cy/TBI or etoposide (Vep/TBI).

**LEUKEMIA WORKING PARTY OF THE EBMT**

**TOTAL BODY IRRADIATION AS CONDITIONING FOR ADULTS WITH CYCLOPHOSPHAMIDE**

observed had no impact on OS, NRM, CI of Relapse and acute and chronic GvHD. Setting of a MA conditioning with PT-CY the real degree of HLA mismatching was 6.6%; no correlation was observed with the amount of HLA mismatch in GvHD (12.6% vs 3-4 mm:58.8%, p=0.58 and 0-2 mm:18.2% vs 3-4 mm:19.1%, p=0.93, respectively). Considering only GVH directed mismatches, no difference was highlighted between low or high HLA mismatch burden in cumulative incidence of grade II – IV aGVHD (12.6% vs 3-4 mm:58.8%, p=0.58 and 0-2 mm:18.2% vs 3-4 mm:19.1%, p=0.93, respectively). Considering only GVH directed mismatches, no difference was highlighted between low or high HLA mismatch burden in cumulative incidence of grade II – IV aGVHD (12.6% vs 3-4 mm:58.8%, p=0.58 and 0-2 mm:18.2% vs 3-4 mm:19.1%, p=0.93, respectively).

**S797 CYCLOPHOSPHAMIDE VERSUS ETOPOSIDE IN COMBINATION WITH TOTAL BODY IRRADIATION AS CONDITIONING FOR ADULTS WITH PH(-) ALL UNDERGOING ALLO-HCT: A STUDY FROM THE ACUTE LEUKAEMIA WORKING PARTY OF THE EBMT**


**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.

**S798 ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA WITH DELETION 5q OR MONOSOMY 5: A STUDY FROM THE ACUTE LEUKAEMIA WORKING PARTY OF THE EBMT**


**Background:** Acute myeloid leukemia (AML) is mainly defined by the presence of determined poor-risk cytogenetic abnormalities and is a standard 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 long-term effects on 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)).

**Results:** Five hundred and one pts, 21% of them with secondary AML, have been included in this study. The incidence of -5q/-, with -5q/- reported to the EBMT registry as having their first SCT between 2000 and 2015.

**Methods:** Adult patients with Ph-negative AML (n=1498) treated with alloHCT from either HLA-identical sibling (n=696) or unrelated donor (n=802), in CR1 (n=1186) or CR2 (n=312), between year 2000 – 2015, were included in the analysis. Peripheral blood was used as a source of stem cells in 62% of the patients. Conditioning was myeloablative in all cases (the median TBI dose was 12 Gy); 1346 patients were treated with Cy/TBI while 152 patients with Vep/TBI. Patients in the Vep/TBI group were younger (median 28 y. vs 30 y., p=0.04), treated in more 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 an univariate analysis, as compared to Cy/TBI, 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=0.001) 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 siblings vs unrelated donor transplants (HR=1.47, p=0.01) and donor/recipient gender combination other than female/male (HR=1.80, 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 without CK, MK or abn(17p). Group 2 (CK) included pts with -5/5q- and CK but no MK or abn(17p) (N=90). Group 3 (MK) included 169 pts with -5/5q- and MK but no abn(17p). Finally, group 4 (17p) included pts with -5/5q- and abn(17p) (N=193). The 4 groups were quite similar in terms of characteristic. The 2-year probability of LFS was 39% for group 1, 25% for group 2, 20% for group 3 and only 13% for group 4 (p<0.001). 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 CK but no MK or abn(17p) on SCT outcome. Future efforts should be focused on this subgroup in order to improve their outcome.

Biomarkers in ALL

S799

IDENTIFICATIONS OF NOVEL RECURRENT PU.1 FUSIONS WITH HIGHLY AGGRESSIVE PHENOTYPE IN PEDIATRIC T CELL ACUTE LYMPHOBLASTIC LEUKEMIA


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Background: T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) accounts for 10% to 15% of newly diagnosed cases of childhood acute lymphoblastic leukemia (ALL), arising from the malignant transformation of hematopoietic progenitors primed toward T cell development, as result of a multistep oncogenic process. However, since the prognostic significance of these genetic alterations in pediatric T-ALL is not clear, genetic basis which contributes aggressive phenotype or progression of pediatric T-ALL is still to be elucidated.

Aims: To discover driver genetic events, which involved in the aggressive phenotype of pediatric T-ALL and to identify its novel prognostic markers, we performed integrated genetic analysis in a large cohort of T-ALL case.

Methods: Our cohorts included samples from Tokyo Children’s Cancer Study Group (TCCSG) and Japan Association of Childhood Leukemia Study (JACLS). Whole transcriptome sequencing (WTS) was performed in 123 cases. Whole transcriptome sequencing (WTS) was performed in 123 cases.

Results: Representative recurrent fusion genes were as follows, S1L-TAL1 (n=25), MLL-ENL (n=5), PICALM-MLLT10 (n=5), and NUP214-ABL1 (n=2).

Intriguingly, novel recurrent in-frame PU.1 fusions (STMN1-PU.1 n=2; TCF7-PU.1 n=5) were detected, and RT-PCR analysis in additional 60 cases revealed other 2 TCF7-PU.1 fusions. Thus, PU.1 fusions accounted for 4% of pediatric T-ALL/LBL.

Expression data of WTS revealed cases with PU.1 fusion showed significantly higher expression of PU.1 compared to cases without PU.1 fusion, implicating that aberrant high expression of PU.1 involved in leukemogenesis.

Using consecutive two-step unsupervised consensus clustering, we obtained 5 stable clusters. Among these, 4 clusters largely recapitulated distinct T-ALL subtypes characterized in previous studies by an early T-cell precursor (ETP) signature (ETP-ALL), 2 clusters of high TAL1 expression (TAL1-RA and -RB-ALL) and mutually exclusive expression of TAL1, and TAL3 (TAL-related-ALL). However, the remaining one was newly identified and exclusively consisted of the 7 PU.1 fusion-positive cases. Compared to ETP-ALL, these PU.1 fusion cases typically showed a reduced expression of the phase I genes implicated in early T-cell development, except for PU.1, which was ectopically up-regulated by the relevant gene fusions. All cases with PU.1 fusion were grouped into PU.1 high cluster. Moreover, PU.1 high cluster had distinct genetic features with mutations of transcription factors, such as GATA3, RUNX1, and EVI6. Of note, significant patient outcome was confirmed by multivariate analysis in cases with PU.1 high cluster (p<0.048). Consistently, we defined PU.1 overexpression cases as outliers of PU.1 expression, which resulting in extremely poor prognosis (3-year OS 21%, log-rank p=6.9 x10^-7).

Summary/Conclusions: PU.1 fusions expressing cells expanded and they remained at an immature stage, implicating a potential leukemogenic activity of these fusions. Not only the cases with PU.1 fusions, but also the cases with
high PU.1 expression without fusions showed extremely poor prognosis, suggest- 
gig the prognostic value of abeant PU.1 expression in pediatric T-ALL. Although it remains unclear, why cases with PU.1 fusions/high PU.1 expression have a poor prognosis, our results indicate that these cases are genetically distinct subgroup from other pediatric T-ALL.

S801
MULTI-CENTER VALIDATION OF STANDARDIZED NGS ASSAYS FOR RARRANGED IG / TR MARKER DETECTION IN ACUTE LYMPHOBLASTIC LEUKEMIA – A REPORT OF THE EUROCLONALITY-NGS CONSOR- 
TIAL
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S802
POST-INDUCTION MRD PREDICTS HIGH RELAPSE RISK FOLLOWING REDUCED INTENSITY CONDITIONED ALLOGENEIC STEM CELL TRANS- 
PLANTATION: A PROSPECTIVE STUDY OF ADULT ALL (UKALL14.ISRCTN 66541317)
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Background: Reduced intensity conditioned allogeneic haematopoietic stem cell transplant (RICalloHCT) enables HCT to be performed in older patients. The UK NCRI UKALL14 study of adult acute lymphoblastic leukaemia (ALL) considers patients ≥41 years “high risk” and recommends a RICalloHCT where there are high quality donors. Other “high risk” factors are high WBC at presentation, t(8;22), t(4;11), hypodiploid/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 myeloablated 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.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n=115</th>
<th>Disease characteristics</th>
<th>n=115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (median range)</td>
<td>49 (20-60)</td>
<td>6-ALL</td>
<td>90 (71)</td>
</tr>
<tr>
<td>Preexisting BCR/ABL1</td>
<td>86 (75.7%)</td>
<td>7-ALL</td>
<td>72 (63%)</td>
</tr>
<tr>
<td>NOS (%)</td>
<td>61 (53)</td>
<td>ALL</td>
<td>55 (48)</td>
</tr>
</tbody>
</table>
| Male | 54 (47) | Leukemic | 6 (5)
| Female | 60 (52) | Ph’negative | 49 (43)
| Blastic morphology | 40 (34.8) | Complex karyotype | 32 (28)
| Medical adjuvant | 50 (43.0) | Fludarabine-based | 43 (37.3)
| Medical adjuvant | 56 (48.3) | EKALLO intermediate risk group N (% | 48 (41.7)
| MRD at Induction (median range) | 6 (0-40) | Standard | 62 (53.8)
| MRD at Induction (median range) | 6 (0-40) | High-risk | 35 (30.4)
| MRD at Induction (median range) | 6 (0-40) | Unknown | 10 (8.7)

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 55.8% respectively. By contrast, in the 38 of 115 patients with positive MRD after induction, OS and EFS were 40.6% and 28.4% respectively. Twenty eight of the 115 patients relapsed, with a 2 year actuarial relapse risk of 31.5% (22.2-43.5). We assessed the association of the following factors; age, sex, immunophenotype, presenting WBC, BCR/ABL1, other cytogenetics, post-induction MRD and donor type with the risk of relapse. Among this population of high risk patients, post-induction MRD was the only independent prognostic factor for relapse (univariable HR: 3.82 (1.59-9.16), p = 0.001 (see Figure 1) and multivariable HR: 4.14 (1.61-10.65), p = 0.003). The relapse rate of the MRD+ patients was 57.2% at 2 years post HCT.

Figure 1.

Summary/Conclusions: The 2-year OS of 62.5% in UKALL14 participants over 41 years of age 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.

S803

T-CELL RECEPTOR B REPertoire characteristics in Relapsed/ Refractory B-Cell Precursor Acute Lymphoblastic Leukemia on Blinatumomab Treatment

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Background: Blinatumomab (Blin) is a bispecific monoclonal antibody, activating autologous effector T-cells and redirecting them against CD19-positive malignant cells. This leads to polyclonal effector T-cell expansion which is the necessary component of its antitumour mechanism. Recent reports indicated promising antitumour activity of Blin in relapsed/refractory (rr) B-cell precursor acute lymphoblastic leukemia (BCP-ALL). However, approximately half of these patients do not achieve minimal residual disease (MRD) response. Thanks to recent advances in next generation sequencing (NGS) of immunoglobulin and T-cell receptor gene rearrangements the deep and comprehensive evaluation of expanded T-cell repertoire on Blin treatment is now possible.

Aims: To compare the differences in TRB repertoire diversity and composition between two groups of patients with rr ALL: 1) responders: reaching MRD negativity at the latest at day 29 of 1. Blin cycle (C1D29), and 2) persisters: with quantifiable MRD positivity (>0.01%) at C1D29, or with MRD >1% at cycle 1 day 15 (C1D15) if C1D29 sample is not available.

Methods: We used NGS to investigate TRB repertoire in bone marrow samples (114× at time of screening (scr), 74× C1D15, 59× C1D29) of 114 rrPh-negative BCP-ALL patients (median age: persisters 47; responders 42; p-value=0.81). Patients received Blinatumomab within the phase II trial (MT103-211). Sequencing libraries were prepared using 100ng of DNA via 2-step PCR and sequenced on the Illumina MiSeq (2 x 250bp) with a median coverage of 117,563 reads (range 59,512 - 447,767 reads) per sample. In the first PCR virtually all TRB rearrangements present in the investigated sample were amplified using universal V(D)- and J-regions primers. In the second step, sequencing adaptors and sample-specific barcodes were added. Annotation of V(D)- and J-regions of TRB sequences was performed using ARRestInterrogate (Bystrý, Bioinformatics, 2016). Diversity of TRB repertoire within patient groups and time points was expressed as the Shannon index, using the R-package vegan. Analysis of variance was employed to assess statistically significant differences in diversity between groups and time.

Results: Diversity of TRB repertoire (Figure 1) was significantly higher in responders compared to persisters at time of scr (p=0.02) and at C1D29 (p=5.47E-6). Patients in the persisters group had significantly higher blast counts, which is in accordance with previously published data (Topp, The Lancet Oncology, 2015). The increase of diversity between scr and C1D29 of Blinatumomab treatment was sharp and highly significant in responders (p=3.96E-6), but not statistically significant in persisters (p=0.4).
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 FEBRILE NEUTROPENIA IS SAFE AND REDUCES EXPOSITION TO ANTIBIOTICS (HOWLONG RANDOMIZED TRIAL)

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Background: In neutropenic patients with unexplained fever the classical approach is maintaining the empirical antibacterial therapy (EAT) until neutrophil recovery. This strategy may result in unnecessarily prolonged EAT favoring bacterial resistance, organ toxicity and damage to microbiota. Nevertheless, the available scientific evidence supporting the alternative approach of stopping EAT before neutrophile recovery is moderate.

Aims: To investigate if a clinical approach (based on apyrexia and clinical recovery) is better than and as safe as the standard criteria (recovery from neutropenia) to decide the discontinuation of EAT.

Methods: After local Ethical Committee approval, a randomized, controlled, multicenter, open-labeled phase IV clinical trial was performed (EudraCT: 2011-005152-34). Study period: May-2012 to May-2016. Inclusion criteria: a) Adult patients (≥18 years); b) Hematologic malignancy or autologous or allogeneic hematopoietic stem cell transplantation (SCT) recipients; c) High risk febrile neutropenia (FN) d) Informed consent signed. Exclusion criteria: etiological diagnosis of FN. Patients were randomized 72 hours after fever onset to: 1. Experimental group (EG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h (independently of neutrophil count) or 2. Control group (CG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h, plus c) >0.5x106/L neutrophils. Follow-up: 28 days from EAT. Primary (efficacy) end-point was number of EAT-free days. Secondary (safety) end-points were total days of fever and crude mortality.

Results: One hundred and fifty seven patients were included (EG 78 and CG 79). There were no differences in baseline characteristics or clinical presentation between groups. The most frequent underlying conditions were induction/re-induction chemotherapy for acute leukemia (n=42; 26,7%), autologous SCT (n=42, 45,8%), and allogeneic 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>EG (n=78)</th>
<th>CG (n=79)</th>
<th>p</th>
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<tbody>
<tr>
<td>Days of neutropenia</td>
<td>14 (5.5-24)</td>
<td>11 (8.2-21)</td>
<td>=ns</td>
</tr>
<tr>
<td>Days of fever</td>
<td>7 (2-28)</td>
<td>7 (2-28)</td>
<td>=ns</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>18 (12.3-215)</td>
<td>16 (9.7-20.2)</td>
<td>0.047</td>
</tr>
<tr>
<td>Per protocol population</td>
<td>EG (n=68)</td>
<td>CG (n=68)</td>
<td></td>
</tr>
<tr>
<td>Days of fever</td>
<td>4 (1-14)</td>
<td>5 (2-28)</td>
<td>=ns</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>19 (14-22)</td>
<td>15 (9.7-20)</td>
<td>0.02</td>
</tr>
<tr>
<td>Modified per protocol population*</td>
<td>EG (n=36)</td>
<td>CG (n=36)</td>
<td></td>
</tr>
<tr>
<td>Days of fever</td>
<td>3 (1-7.2)</td>
<td>4 (1.5-7.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>ITT: Intention to treat; EAT: empirical antimicrobial therapy; EG: experimental group; CG: control group; IQ range: Interquartile range; *EAT-free days: days of follow-up (28) - days of EAT. Patients in which clinical recovery and neutropenia recovery did not match.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 LYMPHOUCYTIC LEUKEMIA: A RANDOMIZED STUDY DONE 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, 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 opsonophagocytic assay (OPA) and enzyme-linked immunosorbent assay (ELISA).

Results: PCV13 elicited a superior immune response than PPSV23 in 10/12 serotypes one month after vaccination and in 5/12 serotypes six months after vaccination, measured as OPA geometric mean titers (GMTs). Geometric mean concentrations of serotype-specific IgG antibodies elicited by PCV13 as measured by ELISA, were higher than those elicited by PPSV23 in half of the common serotypes, both after one and six months. The proportion of patients with good response (defined as response in 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 these serotypes, OPA GMTs were lower in 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 a conjugated pneumococcal vaccine is better than that of the traditional polysaccharide vaccine, 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 early 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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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 2007-Mar 2016). The 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 prospective validation set, 100-day and 2-yrs IRM were 7% (95% CI 3-14) and 14% (95% CI 8-26) 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=0.003). Out of a total of 125 infection-related deaths, 94/129 (73%) were attributed to bacteria, 22/129 (17%) to viruses, 11/129 (8%) to fungi and 2/129 (8%) 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 in on the way for the external validation of these results.

S807
LETERMORIV FOR PREVENTION OF CYTOMEGALOVIRUS INFECTION IN ADULT CMV-SEPOSITIVE RECIPIENTS OF ALLOGENEIC 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. LET 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% haploidentical and 4% cord blood. Study arms were balanced. Subjects began study drug a median of 9 days post-HCT; 37% had engrafted prior to start. Of 495 treated subjects with undetectable CMV DNA at randomization, fewer subjects developed CS-CMV or were considered failures in the LET arm (122/325, 38%) compared to placebo (103/170, 61%; p<0.0001) by Week 24 post-HCT. Figure 1 shows the time to CS-CMV analysis. The most common AEs (LET, placebo) were GVHD (39%, 39%), diarrhea (26%, 25%), and nausea (27%, 23%). More frequent vomiting (19%, 14%), edema (19%, 13%), 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.

Support: Jazz Pharmaceuticals.

Efficacy and Safety of Defibrotide to Treat Hepatic Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome Post-Chemotherapy: A Post Hoc Analysis of Final Data of an Expanded-Access Protocol

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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 (50%), 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 of age) 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 day 18.5 due to a block in tissue-specific aération in neurons and T-cells cause 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 erythrocyte 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 C57BL6 female mice were reconstituted with bone marrow cells from Gpx4fl/fl; Rosa26-CreERT2or Gpx4wt/wt; Rosa26-CreERT2 and allowed to recover for 8 to 10 weeks. GPX4 deletion in the hematopoietic system was induced by feeding tamoxifen citrate for 3 weeks and blood and organs were drawn at 3 and 6 weeks after terminating the tamoxifen-containing diet. Erythroid cells have been analysed in FACS. Serum iron levels have been assessed using the SFBC and UIBC iron kits (Biolabo). Gene expression analysis has been performed using SYBR-green qRT-PCR. Circulating Hepcidin has been measured with a colorimetric assay. All animal experiments were approved by and conducted in compliance with institutional guidelines. In vivo studies. RESULTS: Compared to Gpx4fl/fl; 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. Reticulocytes and reticulocyte count measurement revealed a strong increase in this population, suggesting that the erythropenia could be due to a block in the reticulocyte maturation. Reticulocytes FACs characterization revealed a shift towards a more immature population while electron microscopy analysis showed an accumulation of unphagocytosed vesicles containing remnants of mitochondria. Analysis of the spleen revealed extramedullary erythropoiesis. The anaemia and the erythropenia trigger a hypoxic signature hallmarking 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 downregulation 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 MODULATION OF THE INTRACELLULAR IL-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 hypoferrremia, with consequent iron-restricted erythropoiesis and 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 the anemic state with different insilico, invitro and invivo studies. Methods: A systematic 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 turpentine 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 Invivo results showed that co-administration of GDP and ferrous sulphate (FeSO4) significantly improved the turpentine-induced anemic state with increase in haemoglobin level (Figure 1B).

Figure 1.

Summary/Conclusions: AI as 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 regime 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: RAPID 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 due to large deletion mutations in SEC23B gene. More than 300 CDAII cases and 80 causative mutations have 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 demonstrated promising preclinical and clinical 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.

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Madrid, Spain, June 22 – 25, 2017
**Aims:** The main aim of our study is to assess the effects of RAP-011 on different cell types of CDAII treated with ESA.

**Methods:** We measured circulating GDF11 levels in CDAII patients and healthy controls (HC) by western blot (WB). To assess the effectiveness of RAP-011 (provided by Celgene Corporation) in vitro, we established two different cellular model systems: (i) K562 cells stably silenced SEC23B by shRNA carried in lentiviral vector (LV) (ii) K562 ESAs stably overexpressing SEC23B-WT and the two variants, R14W and E109K. In vitro treatment has been performed at 0, 3, and 6 days of erythroid differentiation by 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 overexpression in SEC23B silenced K562 cells, at 3 and 6 days of erythroid differentiation. The given network was not fully connected, we performed pairwise comparisons on the four subnetworks with 2 treatments each. On the one hand, reticulo-endothelial macrophages are central for the regulation of iron homeostasis. The phagocytosis and degradation of senescent red blood cells (RBC) by macrophages enable efficient recycling of iron and the maintenance of systemic iron balance. On the other hand, iron exerts multiple effects on macrophage polarization and functionality. Macrophages exhibit a remarkable functional plasticity, reflected in their capacity to integrate diverse signals from the microenvironment and acquire distinct phenotypes. Macrophage polarization has been shown to dictate the expression of iron-regulated genes and determine cell iron handling. Authors showing evidence that iron availability itself has significant effects on immune effector functions and macrophage polarization. However, it is still unclear how different iron sources and acquisition pathways affect macrophage phenotypes.

**Methods:** To investigate this aspect, we analyzed both in vivo and in vitro, and compared the phenotypic switching of macrophages induced by different iron sources, including heme and iron, as well as hemolitic or intact RBCs.

**Results:** Hemolitic RBCs, free heme and iron-dextran treatment in mice shape macrophage polarization towards an M1-like pro-inflammatory phenotype. Splenic and hepatic macrophages from treated mice show an increased expression of pro-inflammatory cytokines (IL-6, TNFα), and a reduced expression of anti-inflammatory markers (M2). Moreover, in these cells, the expression of M1 markers such as CD86 and CD80 is strongly increased, whereas the expression of M2 markers such as CD206 and CD163 is significantly suppressed. Consistent results have been obtained treating bone marrow-derived macrophages with hemolytic RBCs, free heme and Fe-NTA. Importantly, the addition of the heme scavenger hemopexin and the iron carrier transferrin or the chelator deferoxamine fully abolish the ability of free heme and iron to trigger M1 polarization. On the contrary, RBC transfusions in mice shape macrophages towards an M2-like anti-inflammatory phenotype. After three transfusions, serum iron and hepcidin levels significantly rise, and tissues as well as macrophages are heavily iron loaded. Macrophages show a drastic suppression of M1 markers and inflammatory cytokines, and induction of M2 markers. Interestingly, repeated transfusions result in extensive macrophage death and new macrophage recruitment in both liver and spleen.

**Summary/Conclusions:** Collectively, these results suggest that the source and route of iron acquisition have a key role in shaping macrophage phenotype, and demonstrate a dynamic role of iron overload in determining macrophage polarization and function. When iron is provided in the form of free heme or non-transferrin-bound iron, it exerts a clear pro-inflammatory effect on macrophages; whereas when provided via a controlled physiological acquisition pathway such as erythropoietin, it dampens macrophage immune effector functions, being its clearance activity more active.

Figure 1.

**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.

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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-B0/B0 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 autologous 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 HbAT87Q 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 3.2 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 HbAT87Q over time.

Results: As of March 1, 2017, two 20-year-old females with β0/βE genotypes have been treated with LentiGlobin DP in the Northstar-2 trial. The DP VCN was 2.9 and 2.4 copies per diploid genome, respectively. Outcomes in all evaluable patients will be presented.

Summary/Conclusions: Results from the Northstar-2 study will provide data on safety and demonstrate the extent to which an increase in LentiGlobin DP VCN yields normalization of total Hb and consistently achieves transfusion independence in patients with TDT of non-β0/β0 genotypes. Optimizing DP VCN has the potential to improve outcomes across all TDT genotypes treated by investigational LentiGlobin gene therapy.

S815

CIS IS A POTENT CHECKPOINT IN NK CELL ANTI-LEUKEMIA IMMUNITY

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Background: The detection of leukemia by natural killer (NK) cells is controlled
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, structural biology, kinase enrichment and activity assays, NK cell in vitro analysis, biochemistry and de novo/experimental tumour/leukaemia in vivo models.

Results: We identified cytokine-inducible SH2-containing protein (CIS, encoded by Cish) as a critical negative regulator of IL-15 signaling in NK cells. Cish was rapidly induced in IL-15-activated NK cells and deletion of Cish rendered NK cells hypersensitive to IL-15, as evidenced by enhanced proliferation, survival, IFN-gamma production and cytotoxicity toward tumors. This was associated with increased JAK-STAT signaling in NK cells in which Cish was deleted. Correspondingly, CISH interacted with the tyrosine kinase JAK1, inhibiting its enzymatic activity and targeting JAK for proteasomal degradation. Cish/-/- 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 CISH 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


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 ALCL and ALC patients although duration of responses is short in the majority of cases. Here, we developed a refined second generation 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 engagement and persistence of CD30-CAR T cells after transfer, and we have included an EGFRt 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-EGFRt CAR was generated using a scFv that recognizes a tumor-cell membrane-proximal epitope of CD30 protein (Nagata S et al. Clin Cancer Res, 2002). 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 1:0.5 effector/target (E/T) ratio, and the tumor cell death induced by flow cytometry analysis of the membrane antigens (IFN-y and IL-2) were analysed at 24 hours in a 5:1 E/T ratio culture using Luminescence technology.

Results: Two TSCM were the most prevalent T-cell subset at day 10 of culture, representing 84 ± 3% of total cells, and the CAR expression by CD30-CAR in expression was in these cells was 76.9 ± 1.0% in CD4+ TSCM and 77.3 ± 2.0% in CD8+ TSCM. Although CD30-CAR protein was detected in a fraction of activated T cells in culture (CD4+ T cells: 32.4 ± 2.1%; CD8+ T cells: 59 ± 4.3%), lentiviral transduction of TSCM with our CD30-CAR did not compromise their ex vivo expansion (CD4+ CD30-CAR TSCM: 96.0 ± 3.2 fold expansion; CD8+ CD30-CAR TSCM: 109.0 ± 4.2 fold expansion). CD8+ CD30-CAR TSCM conferred specific cytotcic activity and lysed CD30+ PTCL in a dose-dependent manner (tumor cell death 1:1 ratio: 92.6 ± 2.2% vs 0% with untransduced TSCM; p<0.001), while control CD30+ target cells (Raji) were not recognized. In addition, CD30-CAR TSCM secreted IFN-y and IL-2 after stimulation with Karpas 299 cells (IFN-y: 126.6 ± 18.12 pg/ml with control targets, p=0.002; IL-2: 20.47 ± 2.3 pg/ml with control targets, 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.
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.

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.
Preclinical Combination of a Novel IRE1 RNAse Inhibitor MKC-8866 and Tyrosine Kinase Inhibition Acts Synergistic in Acute Lymphoblastic Leukemia

M. Vieri1, A. Salimi1, J.B. Patterson2, A. Samali3, E. Cheve4, T.H. Brümmendorf1, I. Appelmann1, B. Kharabi1

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Background: The role of the Unfolded Protein Response (UPR) in BCR-ABL+ ALL is controversial. Previous studies were performed using either IRE1 or BCL-2 ALL cell lines and did not compare their responses side by side.

Aims: To study the effect of IRE1 inhibition alone and in combination with Tyrosine Kinase Inhibitors (TKI, using Imatinib or Nilotinib) on BCL-2 ALL cell lines, SUP-B15 and TOM-1.

Methods: Cells were treated with different concentrations of IRE1 inhibitor or TKI, alone or in combination, for 72 hours. Cell survival and viability were evaluated by CellTiter-Glo assay.

Results: IRE1 inhibitor MKC-8866 (MKC) was effective in terms of cell death, and its combination with the TKI resulted in improved cell death compared to single treatments. The most promising combination was 30µM MKC and 10µM IM, which showed a synergistic effect.

Overall, our data demonstrate that simultaneous inhibition of IRE1 and BCL-2 ALL cell lines could be a promising therapeutic strategy for this disease.

E821 Critical Role for Notch Signalling in B-Cell Precursor Acute Lymphoblastic Leukemia (B-ALL) Drug Response

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Background: Notch signalling is known to be involved in B-ALL pathogenesis. However, its role in drug resistance and treatment response is still unclear.

Aims: To investigate the role of Notch signalling in B-ALL drug response.

Methods: E821 vs. B-ALL xenograft models were established using the B-ALL line RS4;11. Flow cytometry and western immunoblotting were used to study the role of Notch signalling in drug resistance.

Results: Notch-3 and Notch-4 were found to be activated in B-ALL xenografts. Treatment with Notch inhibitors selectively reduced tumour growth compared to standard chemotherapy. This suggests that Notch inhibition may be a potential therapeutic strategy for B-ALL.

Conclusions: Notch signalling plays a critical role in B-ALL pathogenesis and its inhibition could be a promising therapeutic target.

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: Chromosomal abnormalities are common in B-ALL and are used to predict clinical outcomes. However, the wide spectrum of abnormalities makes it challenging to develop targeted therapies.

Aims: To develop a high-throughput, high-resolution and comprehensive disease-relevant CNA profiling approach applicable to all subtypes of pALL.

Methods: A novel dMLPA (digital MLPA) technique was developed which combines the advantages of MLPA and next-generation sequencing (NGS), massively improving the number of simultaneously analyzable genomic loci. The number of simultaneously analyzable genomic loci is limited to 55-60.

Results: dMLPA showed a sensitivity of 99.3% for detecting all CNAs, and was able to detect subclonal aberrations with an improved efficacy and confidence as compared to conventional NGS. It was also able to detect subclonal aberrations with an improved efficacy and confidence as compared to conventional NGS.

Conclusions: dMLPA is a robust, fast and cost-effective technique that can be used to detect subclonal aberrations in B-ALL and may provide insights into the heterogeneity of this disease.

E819 Preclinical Combination of a Novel IRE1 RNAse Inhibitor MKC-8866 and Tyrosine Kinase Inhibition Acts Synergistic in Acute Lymphoblastic Leukemia

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Conclusions: dMLPA is a robust, fast and cost-effective technique that can be used to detect subclonal aberrations in B-ALL and may provide insights into the heterogeneity of this disease.
NOD/Shi-scid/I.L-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 derived 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 Dasatinib regulates the expression of Notch receptors. This down regulation was also observed in human CD19+ blast cells isolated from bone marrow of recipient mice treated with Ara-C compared to cells isolated from not treated mice. In addition, Notch inhibitors significantly improved in vitro the cytotoxicity of Ara-C toward B-ALL cells. Finally, we evaluated the administration to mice of a pan Notch inhibitor, i.e. the GSI XII, significantly lowered the CD19+ leukemic burden in the bone marrow of recipient mice, potentiating anti leukemic effect of Ara-C.

Summary/Conclusions: In this study we used both in vitro and in vivo assays to highlight the prognostic value of Notch expression in B-ALL, as well as its critical role in B-ALL cell survival and response to chemotherapy. We also demonstrated that Notch inhibitors were able to improve Ara-C-mediated reduction of blast cells in bone marrow, revealing that Notch signalling is a possible therapeutic strategy to eradicate minimal residual disease in B-ALL.

E823

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-18-181b1 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 over-expression in murine 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 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+W T-ALL cells we observe an increase in c-Myc expression.

Consistent with these results, NOTCH1-WT NRARP overexpressing cells are more sensitive to JQ1, a small-molecule bromodomain inhibitor that targets c-Myc. NRARP is known to positively regulate LEF1, a DNA binding transcription factor acting downstream of WNT. Thus we sought to investigate the impact of this interaction in the signaling pathway. Very interestingly, our results show that in NOTCH1-mutant cell lines 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 CD27/CD44low-neg immunophenotype. During diagnostic immunophenotypic analysis 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 hyperdiploid cytogenetics, 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 hyperdiploid ETV6/RUNX1+ cases have a unique genomic landscape.

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 CD27/CD44low-neg B-other ALL cells and compared it with the expression of NRARP in human thymocytes.

Results: Consistent with these results, microarray data from all 17 B-ALL cases were combined with data from an independent Italian cohort of 291 B-ALL cases (including ETV6/RUNX1-positive, BCR/ABL1-negative, TCF3/PBX1-negative, 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 CD27/CD44low-neg 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) uniparental disomies (5 to 27 per case) and point mutations (10 to 41 per case) in all 7 cases and 3 in-frame fusions transcripts 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 ARP221 was found in 3 cases, and the deletions of PAX5, ATR10A, BTG1 and the gain of RUNX1 were found in 2 cases each. The ARPP21 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.

Summary/Conclusions: We showed that similarly to ETV6/RUNX1-positive ALL, ETV6/RUNX1-like ALL is also associated with CD27/CD44low-neg immunophenotype. We identified deletion of ARP221 to contribute to the specific genomic profile of ETV6/RUNX1-like ALL in addition to lesions of ETV6
and IKZF1. 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 TPOG-ALL-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-AF10 transcripts were detected by RT-PCR assays. RQ-PCR with TaqMan assays were used to measure the expression of HOX11, TAL1, and LYL1 oncogenes expressed as normalized copy number (NCN) to ABL internal control gene. TAL1 overexpression was defined as NCN > the lowest level of SIL-TAL1 positive patients. Overexpression of HOX11 and TAL1 and LYL1 was defined as NCN > the upper limits of the 95th normal bone marrow controls.

Mutations of NOTCH1, FBXW7, PHF6, JAK1, JAK2, RUNX1, WT1, NRAS, and KRAS genes were analyzed by PCR-based assays followed by direct sequencing. P16 deletion was determined by RQ-PCR or multiplex ligation assay amplification (MLPA), PTEN and PHF6 deletions, MYB duplication and NUP214 deletion were detected by real-time PCR and immunoblotting, respectively. Downregulation of PRDX1 was estimated by Western blotting.

Results: The frequency of SIL-TAL1 fusion transcript was 16.2%, MLL-rearranged 5.1%, CALM-AF10 1.0%, and no NUP214-ABL1. The frequency of NOTCH1 mutations was 46.9%, FBXW7 13.0%, RUNX1 5.2%, WT1 6.3%, NRAS 6.2%, KRAS 2.1%, and no JAK1 or JAK2 mutations. P16 deletion was present in 56.2%, PTEN in 11.1%, PHF6 deletion/mutation in 13.4%, and MYB duplication in 4.8%. Overexpression of TAL1 was present in 46.5%, 22% for LYL1, and 9% for HOX11. The correlation among the genetic alterations showed that LYL1 overexpression occurred more frequently in P16 wild-type compared with P16-deleted patients (P=0.003) and absence of SIL-TAL1 transcript was significantly associated with wild-type HOX11 overexpression (P=0.018). A comparative assessment 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=0.001) and OS (P=0.001). Status of other gene mutations, deletion or duplication did not influence the RFS or OS. TAL1 overexpression predicted a higher risk of relapse (37% vs 21%, P=0.006), an inferior RFS (P=0.002) and OS (P=0.025) whereas HOX11 or LYL1 overexpression had no prognostic impact. By multivariate analysis, NOTCH1, PTEN, and PHF6 mutations/statistical significance and statistical independence for an independent predictor of OS (HR 0.167, P=0.112), PHF6 deletion/mutation was an independent unfavorable predictor for OS (HR 5.096, P=0.006), and PTEN deletion was also an independent predictor for both RFS (HR 29.493, P=0.007) and OS (HR 15.830, P=0.003). TAL1 overexpression was an independent risk factor for both RFS (HR 3.989, P=0.014) and OS (HR 2.701, P=0.047).

Summary/Conclusions: The present study showed that LYL1 overexpression was negatively associated with SIL-TAL1 or P16 deletion. PHF6 deletion/mutation, PTEN deletion, and TAL1 overexpression were the independent predictors of adverse outcomes. (Grants support: CORPG3C0201, MHM-E-105-09, NSC-101-2314-B-195-004-MY2, and Tory 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 disrupted in parallel. Loss of one copy (haploinsufficiency) of a neighbouring gene that is essential for the survival of the cancer cells may constitute potential therapeutic targets in that the cancer cells may be selectively sensitive to further suppression of the function of that gene. Identifying such vulnerabilities is one of the current challenges in cancer genomics. We show that vulnerabilities in cancer cells can be identified by analyzing 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 a 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 spanning 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 analysis and by investigation of their known function. The framework thus 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 model 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 in adolescents and adults (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 antioxidants 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 interchange 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 cytoge- netic subgroups of B-ALL: BV-173 (BCR-ABL), SEMK-2 (MLL-4AF4) and NALM-6 (t(12;21)(p13;q22);PHF6). ROS levels were measured using E827.

CRISPR v2 plasmid to produce lentiviral vectors encoding PRDX1-specific sgRNA and Cas-9 and used them to generate BV-173 cells with PRDX1 genomic deletion. Proliferation rate was evaluated by trypan blue exclusion method. Cytostatic/cytotoxic effects of TXN-family enzymes inhibitors, such as adenanthin (ADE), auranofin (AUR) and 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 compared to normal B cells isolated from human tonsils (Fig 1A). In agreement 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 both at RNA and protein levels. Genomic deletion of PRDX1 in BV-173 cells leads to suppression of their proliferation rate, comparing to parental cells and cells transduced with mammalian non-targeting sgRNA. These results allow us to suspect that PRDX1 may play growth-supporting role in these cells. Targeting TXN-family enzymes was also performed with the use of various small molecule inhibitors. Both B-ALL cell lines and primary cells are sensitive to PRDX and TXN inhibitors, which reduce cell viability in dose-dependent manner.

Summary/Conclusions: All the above results suggest that targeting TXN antioxidant system may exert desirable anticancer effects in the treatment of B-ALL. Inhibitors of TXN-family enzymes can be considered as putative agents to use in combination with classical drugs and improve existing therapeutic approaches. Further studies are underway.

E828

RNA-BINDING PROTEIN IGF2BP1 PROMOTES SURVIVAL OF ET6V/RUNX1 LEUKEMIA CELLS

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Background: The IGF2 mRNA binding protein 1 (IGF2BP1, other aliases IMP-1 (IMP1), CRD-BP (CRDBP), ZBP-1 (ZBP1), and VICKZ1) belongs to a family of regulatory RNA-binding proteins with an oncofetal expression pattern. IGF2BP1 has also been identified to be exclusively specific for ET6V/RUNX1-positive acute lymphoblastic leukemia (ALL) but biological significance of IGF2BP1 overexpression has not been thoroughly investigated to date (Andersson, Olofsson et al. 2005; Stoskus, Gineikiene et al. 2011). We have recently contributed by reporting that ET6V/RUNX1 transcript is a target of RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL, suggesting a role of IGF2BP1 in ET6V/RUNX1-mediated leukemogenic events (Stoskus, Vaitkevičiūnė et al. 2016).

Aims: To define the biological significance of IGF2BP1 overexpression in t(12;21)(p13;q22) ET6V/RUNX1-positive ALL.

Methods: In this study we have used stable sublines with downregulated IGF2BP1 from our previously published study (Stoskus, Vaitkevičiūnė et al. 2016). Dynamics of viable cell population was assessed by flow cytometry using 7-AAD staining (BD Biosciences) following 72 hrs culture in complete medium. An EdU flow assay (Thermo Fisher Scientific, TFS) was used to assay DNA replication in proliferating cells. Spontaneous and doxorubicin (Doxo), staurosporine (STS), and STAT3 selective inhibitor S3i-201 (all from Santa Cruz Biotechnology) induced cell death rates were determined by Annexin V (TFS) and 7-AAD staining. All samples were analyzed on Accuri C6 cytometer (Accuri Cytometers) using CFlow Plus and FCS Express software (De Novo Software). IGF2BP1, ET6V/RUNX1, and STAT3 RT-qPCR was performed previously (Stoskus, Gineikiene et al. 2011). Statistical analyses performed using GraphPad Prism software (GraphPad Software).

Results: Downregulation of IGF2BP1 by 2-fold have rendered into approximately 2-fold lower population growth rate, increasing levels of spontaneous cell death in dynamics, and modest yet statistically significant attenuation of cell cycle progression (35.13% vs 40.40%, p<0.0001). Data from treatment with 50 nM of Doxo, 250 nM of STS suggest that IGF2BP1 downregulation has no effect on pharmacological effectiveness of these drugs. In contrast, IGF2BP1-downregulated cells are more sensitive to pharmacological inhibition of STAT3 even upon treatment with suboptimal 25 µM concentration of S3i-201. Lastly, we have probed if STAT3 transcript levels could be sustained by IGF2BP1 protein as in agreement with previously reported (Stohr, Kohn et al. 2012) and our unpublished insights from anti-IGF2BP1 RNA immunoprecipitation datasets. Correlation analysis of RT-qPCR data have confirmed these assumptions as downregulation of IGF2BP1 expression have resulted in a decrease of ET6V/RUNX1 mRNA (r2=0.8253, p<0.001, slope 0.9459) and also STAT3 transcript levels (r2=0.7709, p=0.002, slope 0.6436). 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).
suggest that 6-MP inhibits the phosphatidylinositol 3 kinase (PI3K)/ mammalian target of Rapamycin (mTOR) 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 expression of lipostatic genes to sustain cell growth, proliferation, and a key feature of cancer cells. This metabolic switch is regulated by metabolic checkpoints, including mTOR, AMP-activated protein kinase (AMPK) and the oncogenes Myc and HIF-1α.

Aims: Our objective is to study the impact of the antiproliferative molecule 6-mercaptopurine (6-MP) on glucose uptake and on glucose transporters (Glut1 or Glut3, SLC2A1 or SLC2A3) and the impact on clinical outcome.

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 glucometabolism 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 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 inhibits TCA (tricarboxylic acid cycle) and oxPKCS (oxidative phosphorylation). The production of lactate, a marker of aerobic glycolysis, is significantly decreased by 30% after 6-MP treatment for 48 hours, meaning that aerobic glycolysis is also inhibited. However, 6-MP has no effect on glucose uptake or on glucose transporters (Glut1 or Glut3, SLC2A1 or SLC2A3) and the main expression, suggesting that 6-MP metabolic effects are not linked to glucose uptake.

Summary/Conclusions: In conclusion, our findings offer new insights on the cellular effects of 6-MP treatment by promoting an early energetic stress that influence proliferation and raise apoptosis in leukemia T cells. Interestingly, the suppression 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.
Results: With a median follow up of 12.43 [2.4;30.3] months, the median OS of the 31 patients at first relapse was 7.9 months, [2.4;13.8]. The OS of patients at first relapse was significantly lower in those having more than 3 CNA by MLPA (median ≤3 CNA 9.7 months [0-20.7] vs median >3 CNA 4.2 months [0.6-7.8], p=0.042). CDKN2A/B deletion was the most common CNA observed at relapse (16/31, 52%) and most of these deletions were homozygous (12/16, 75%). CDKN2A/B deletions, homozygous or more frequent at relapse (from 8 heterozygous CDKN2A/B deleted patients at diagnosis, 7 became homozygous at relapse, p=0.070). SNAP 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) it was 17.8 CNA (12.6 DEL, 4.2 DUP and 1 LOH) and in second relapse (n=5 patients) was 21 CNA (14.6 DEL, 6.4 DUP and 0 LOH) (p=0.007). All matched diagnosis and first relapse samples (available for 10 patients) showed common CNA. In 6/10 cases some of CNA were retained from diagnosis while others were acquired or lost at relapse (suggesting that most of them are not due to clonal evolution). CNA were also observed in 6/10 patients at diagnosis, acquired new CNA at relapse (indicating an evolution from diagnosis clone) and 1/10 showed the same CNA signature at relapse (suggesting a primary resistance of the diagnosis clone). Gene ontology analysis showed a significant enrichment of gene deletions involving B cell differentiation, activation and proliferation, and regulation of cytokine-mediated signaling pathway at relapse (Benjamini Hochberg test, p<0.01). Table 1 summarizes the frequencies of the most retained or acquired CNA at relapse in at least 4 out of 15 patients. Besides the high genetic heterogeneity observed, some recurrent CNA could be identified such as 9p, 1q, 12q, 22q and 7p deletions and 1q, 8q, 17q, 21+ and t(8;21) duplications. Several recurrent tumor suppressor genes such as TP53, FOXO1, FOXO3 or RB1 were detected in 3 patients.

Summary/Conclusions: BCP-ALL has a high genetic heterogeneity at relapse, with most of the genetic alterations playing important roles for disease progression. This heterogeneity points out the need for search of personalized treatment modalities based on their molecular adaptations. Finally, we intend to focus our efforts on 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
IGF1R/IRS PHARMACOLOGICAL INHIBITION REDUCES CELL PROLIFERATION AND MIGRATION IN ACUTE LYMPHOBластIC Leukemia CELLLS
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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/IGF1R 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 (10µM OSI-906 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 it 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 (GI254C1R/IR 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, remains poorly understood.

Aims: We herein aimed to investigate the impact of the pharmacological IGF1R/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 3.2µM, or with OSI-906 at 0.5, 1, 10 and 50µM, for 24 and 48 hours. After drug exposure, cell lines were evaluated for cell proliferation (Ki-67 assay), migration (Time-Lapse microscopy analysis) and cell adhesion (using human umbilical vein endothelial cells HUVEC monoclayer). Statistical analyses were performed by the ANOVA. Value p<0.05 was considered statistically significant.

Results: NT157 decreases cell viability (p<0.05) over 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 Nalmaw leukemia cells and the human endothelial cell monolayer was not significantly modulated by treatment with both inhibitors.

Summary/Conclusions: The reduction on cell proliferation found during IRS/2 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 IGF1R/IRS1 pathway in the break of the blood-brain barrier.
Aims: This study aimed to determine the regional-specific hospitalization days per patient in the INO-VATE trial.

Methods: Patients receiving study treatment (safety population) and recruited from the US and the EU were included in the analyses. The total number of days hospitalized for each patient was calculated. Hospital days prior to randomization and those after the end of study treatment were excluded. Due to different durations of treatment for InO compared to SOC (median 1 vs 3 cycles), calculations were performed 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.

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<th>InO (N=149)</th>
<th>SOC (N=115)</th>
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<tr>
<td>Mean (Days)</td>
<td>23 (11, 70)</td>
<td>33 (24, 93)</td>
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<tr>
<td>Median (Days)</td>
<td>11 (7, 46)</td>
<td>20 (11, 54)</td>
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<tr>
<td>Hospitalized (%)</td>
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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.

E835

HOSPITALIZATION FOR PATIENTS IN THE U.S. AND EU TREATED WITH INOTUZUMAB OZOGAMICIN VS STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN A GLOBAL PHASE 3 TRIAL

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, with its once a week one-hour infusion schedule, has demonstrated lower hospital utilization, in association with a clinically meaningful improvement in overall survival, high rate of complete remission, favorable patient-reported outcomes (PRO), and generally manageable safety profile versus standard of care (SOC, intensive chemotherapy) for relapsed/refractory acute lymphoblastic leukemia (R/R ALL) in the phase 3 INO-VATE trial.

Aims: This study aims to determine the regional-specific hospitalization days per patient in the INO-VATE trial.

Methods: Patients receiving study treatment (safety population) and recruited from the US and the EU were included in the analyses. The total number of days hospitalized for each patient was calculated. Hospital days prior to randomization and those after the end of study treatment were excluded. Due to different durations of treatment for InO compared to SOC (median 1 vs 3 cycles), calculations were performed for cycle 1 treatment period (randomization to end of cycle 1) and for the entire treatment period (all cycles - randomization to end of treatment).

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E836

NON-INTERRUPTIVE 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 at escalated but non-interruptive treatment with low numbers of allo-HSCT- may be of interest and can provide new insights to the common view.

Aims: to evaluate survival data and risk groups in Ph-neg-BCP-ALL pts in the RALL-study.
Methods: The ALL-2009 (NCT01193933) was initiated in Apr 2009. The treatment plan was identical for all risk groups with allo-HSCT indicated only for patients with MRD-SR (26 months vs 35 months. Median EFS of MRD-HR patients was significantly inferior as compared to MRD-SR & 58% as MRD-HR. Twenty patients relapsed & of them, six died (2 were ETP ALL & 18 non-ETPALL: 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-Meyer curve shown in Figure 1). Interestingly, there was no difference in EFS for MRD-HR <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: Our data demonstrate that non-intensive but non-interruptive treatment with fewer allo-HSCTs is more effective in adult BCP-ALL producing more than 50% OS at 7 years, though the TP 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 cytogenetic markers did not matter. So RALL protocol without intensive highly myelosuppressive consolidation courses and high portion of allogeneic 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. ASH, 2014) showed a positive result only in the low risk patients (Day-29). FC-MRD was more sensitive as compared to EFS. This indicates that the best time for MRD evaluation for the risk stratification in T-ALL is still not clear and need more studies. We investigated the value of post-induction FC-MRD response in an assessment of EFS in childhood T-ALL. It is a first T-ALL MRD study from India.

Methods: We studied post-induction (Day-35) MRD (PI-MRD) & post-consolidation (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 dexaxamethasone 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; MF=4–6). Based on the immunophenotypic criteria, 13 patients were diagnosed as ETPALL & remaining 87 as non-ETPALL type. PI-MRD was positive in 58/100 (58%) with the median level of 0.23% (range, 0.002% to 6%). PI-MRD was not performed in 71.4% (30/42) of Ph-MRD-negative & 1.2% (6/58) Ph-MRD-positive patients. PI-MRD was available in 64 patients (30/42 of PI-MRD-negative & 6/58 of PI-MRD-positive). PI-MRD was positive in 28% (18/64) (median, 0.2% & range, 0.009% to 4%). PI-MRD positivity was significantly high in ETPALL as compared to non-ETPALL (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 ETPALL & 18 non-ETPALL: 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-Meyer curve shown in Figure 1). Interestingly, there was no difference in EFS for MRD-HR <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 ETPALL 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 following chemotherapy is 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
Results: 22 patients were enrolled; median age was 29 years (range 16–79), 12 (55%) patients were male, 6 (27%) patients had previously received an allogeneic stem cell transplant (SCT), the most common disease subtype was pre-B-ALL (15, 68%), and 2 (9%) patients had Ph+ B-ALL. 6 (27%) patients received ≥2 cycles of MOR208 and had a subsequent response assessment. Responses were seen in 2 patients; and included a CR and a CRi, giving an overall response rate of 9%. These 2 patients received extended MOR208 treatment. A further 3 (14%) patients did not fulfill the criteria for PR but did not progress; 16 (73%) patients withdrew before completing cycle 2, in most cases due to progressive disease (PD). The patient in CR met the criteria for allo- genetic SCT, but declined this at the time; response duration was 6 weeks, with progression of PD. The patient with the CRi had a response duration of at least 4 weeks, but discontinued due to a treatment-emergent adverse event (TEAE), sclerosing cholangitis. For 12 out of 13 patients with available data, MOR208 treatment led to a rapid reduction in blast/B-cell counts in the peripheral blood; in most cases a reduction of >90% within 1 week of treatment initiation was seen. IL-15 levels were not significantly elevated. Half maximal Caspase cleavage of Poly-(ADP-ribose)-Polymerase (PARP), Caspase 8 and 3, in the SM-sensitive B-ALL cell lines whereas no Caspase cleavage was detectable in the sensitive T-ALL cell lines following stimulation with different SMs. In addition, the bivalent SMs BV6 and Birinapant more effectively induced cleavage of PARP and Caspases than the monovalent SMs in Reh and UoCB6 cells. In addition, we found that SM-induced cell death in Reh and UoCB6 cells is partially dependent on autocrine TNF-secretion. Interestingly, we identified ALL- SCL cells to die in a TNF-dependent manner, whilst CEM cells die independently of TNF. This strongly suggests that TNF is not the only driver of SM-induced cell death in ALL cells.

Summary/Conclusions: We identified a subset of both, BCP- and T-ALL cell lines to be sensitive to SM-induced cell death with IC50 values below 1 micromol. Monovalent SMs are less effective than bivalent SMs in killing ALL cell lines. SMs induce differential modes of cell death with a variable dependency on autocrine TNF secretion in the sensitive ALL cell lines. In-depth molecular characterization of resistance mechanisms of ALL cells to SM-induced cell death is required to identify patients that will benefit from a SM-based treatment regimen.

E839

SINGLE-AGENT MOR208 IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL): A SINGLE-ARM PHASE II STUDY

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Background: CD19 is a type I transmembrane glycoprotein that is expressed throughout B-cell development until terminal plasma cell differentiation. CD19 is also broadly and homogeneously expressed across different B-cell malignancies, including B-ALL. MOR208 is a CD19 monovalent antibody with an enhanced Fc region, which leads to a potentiation of antigen-dependent cell-mediated cytotoxicity and antigen-dependent cell-mediated phagocytosis. Additionally, it has a high occupancy and safety of single-agent MOR208 in the treatment of patients with R/R B-ALL.

Methods: This is a single-arm phase II study of MOR208 in patients aged ≥16 years with histologically confirmed R/R B-ALL with progression after at least one prior therapy. Patients with Philadelphia-chromosome-positive (Ph+) B-ALL were eligible if they had received ≥2 dose-limiting cycles of a tyrosine kinase inhibitor. MOR208 was administered at 12mg/kg IV, weekly, over 28-28 days, with a loading dose on day 4 of cycle 1. Patients with a partial response (PR) could receive a further 2 cycles of MOR208; patients with a complete response (CR) or CR with incomplete count recovery (CRi) after 2-4 cycles could receive an extended phase of consolidation. The primary endpoint was the overall response rate. The trial was prematurely terminated due to insufficient evidence of single-agent activity leading to slow recruitment.

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/) and is the most common childhood malignancy (Hematol Rep 2014;6:5554; Front Oncol 2014;4:63). ALL has an incidence of 1.2 to 1.4 per 100,000 per year in Europe (BM C Cancer 2015;15:771). 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 data from the phase 1 portion of ZUMA-1, a phase 1/2 trial of KTE-C19 in pediatric and adolescent patients with relapsed/refractory (R/R) ALL.

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 2×106 CAR T cells/kg after low-dose conditioning chemotherapy consisting of cyclophosphamide (900mg/m2 onced) and fludara- bine (25mg/m2/d for 3 days) (CyFlu). The primary endpoint of phase 1 is the safety profile. 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 ≥2×106 CAR T cells/kg. KTE-C19 was successfully manufactur- ed in a centralized, streamlined 6-8-day process for all patients across a wide range of baseline absolute lymphocyte counts (0.21–1.0×109/L), except in 1 patient who had disease progression with white blood cells 150,000/L at apheresis and <0.2% T cells in the apheresis collection. All 4 treated patients had high disease burden with a median marrow lymphocytosis 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 siltuximab 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 infections were observed with KTE-C19 at the 2×106 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 ACRE LYMHPHOCYTIC 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 routinely 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 median follow-up of the final cohort was 36.9 months. MRD evaluation was carried out by 8-color FCM (N=73) and RT-PCR of immunoglobulin heavy chain (IgVH, N=62) or T-cell receptor (TCR, N=3) clonal rearrangements and BCR-ABL (N=24), MLL-AF4 (N=4) and E2A-PBX1 (N=1) fusion genes.

Methods with strongest sensitivity for OS prediction on D26 were RT-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 RT-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 RT-PCR (p<0.01).

Summary/Conclusions: Our analysis has shown both RT-PCR and FCM to be suitable methods for MRD assessment on D26 of induction in adult ALL patients requiring an intensive treatment. Furthermore it seems convenient to take any RT-PCR positivity (even below 1.0×10⁻⁴) 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 RT-PCR as a method of choice for MRD assessment in adult ALL while reserving FCM as a backup method for patients without applicable RT-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.

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 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 duration using survival fits obtained in survival regression 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 versus SOC and their differences are shown in Table. Most gains in LY and QALY for patients treated with InO.

Summary/Conclusions: This analysis taking into account both quality and quantity of life estimates shows that InO offers an average of nearly 2 more years of QALY compared to SOC in R/R ALL, based on higher CR and HSCT rates, “tail-of-the-curve” survival gains, and better QoL. This can help inform patients, physicians and payers in decision making.

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>
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<td>0.13</td>
<td>-0.66</td>
<td>0.65</td>
<td>0.08</td>
</tr>
<tr>
<td>CR</td>
<td>0.25</td>
<td>0.06</td>
<td>0.19</td>
<td>0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>Post-HSCT</td>
<td>0.52</td>
<td>0.82</td>
<td>-0.30</td>
<td>2.20</td>
<td>0.44</td>
</tr>
<tr>
<td>Progression</td>
<td>0.16</td>
<td>0.10</td>
<td>0.06</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>1.48</td>
<td>1.77</td>
<td>-0.29</td>
<td>2.48</td>
<td>0.67</td>
</tr>
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</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-CELL PRECURSOR ACUTE LYMPHOCYTIC LEUKEMIA MINIMAL RESIDUAL DISEASE (MRD) ASSAY WITH STUDY OF ARTIFACTS AND MIMICS

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Supported by MUNI/A/1106/2016 grant of Masaryk University, Czech Republic and the Czech Leukemia Study Group for Life.

haematologica | 2017; 102(s2) | 347

Madrid, Spain, June 22 – 25, 2017
FC-MRD assay with high sensitivity of at least 1 in 10^5 and applicability in >97%

We studied 230 BCPALL MRD samples. FC-immunophenotyping and described their prevalence and immunophenotypic features.

Aims: 1. To study the applicability and sensitivity of a 10-color high event single tube FC-MRD assay for BCPALL; 2. To document the rare BM cellular elements with description of rare BM cellular elements and artifacts causing interference in analysis.

Methods: We studied 230 BCPALL MRD samples. FC-immunophenotyping was performed on Navios flow-cytometer using bulk-lysis-and-stain method and data was analyzed with Kaluza-software. MRD was monitored using 10-color single tube FC-MRD assay including CD45, CD10, CD19, CD20, CD34, CD38, CD58, CD98, CD123 and CD25/CD73 with an additional 4-color nuclear dye (SYTO13) tube. Samples with cluster of ≥20 and ≥2 leukemia associated phenotypes (LAIPs) were called MRD-positive. High number of events were acquired for MRD-assay (1.5 to 6 million). To evaluate the applicability of assay, number of LAIPs were determined in diagnostic and MRD samples. In addition, the frequency and antigen expression pattern of mimics and artifacts were studied.

Results: We studied 230 BCPALL MRD samples. High number of events was acquired for MRD-assay with median-events 342700 (range, 1678800 to 6052800). We determined the limit of detection (LOD=10 events) and limit of quantitation (LOQ=30 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% ,<0.01% ,<0.1%, <1% , <10% and >1% and they were respectively 1.74%, 10.43%, 13.48%, 5.65% and 10.00%. Furthermore, in 24 samples with MRD-positive ≥0.01% and >1.5 million acquired-events, the results were compared between time-gated initial 500000-events and initial 1000000-events and all events acquired. Sixteen samples among these were found to be negative in initial 5000000-events and eight in initial 10000000-events highlighting the importance of acquisition of >1.5 million cells.

We categorized different rare cellular events and artifacts in the following way: 1) CD34+ mature B cells; 2) CD10+ mature B cells; 3) CD73+ mesenchymal stromal/ stem cells and endothelial cells; 4) CD123+ CD19+ 7DC precursors; 5) CD86+ CD58+ B cell precursors (BCP); 6) CD19+ NK cells (Table 1). We also described their immunophenotypic features highlighting the differentiating features from MRD and B cell precursors (Figure 1).

Results: Basal evaluation was performed on 26 SR and 16 HR pts. Three SR pts with severe anemia (<7g/dl and/or 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 LVEF at TP150. The 3 remaining HR pts subsequently recovered in GLS value.

Table 1.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>a. main features of SR/HR ALL n=26</th>
<th>M/F</th>
<th>Age</th>
<th>%</th>
<th>Gls</th>
<th>Lvef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.19±0.08</td>
<td>0.18±0.09</td>
<td>0.13±0.09</td>
<td>0.15±0.10</td>
<td>0.11±0.05</td>
<td>0.10±0.06</td>
</tr>
<tr>
<td>Range</td>
<td>0.00-0.26</td>
<td>0.01-0.28</td>
<td>0.00-0.14</td>
<td>0.00-0.23</td>
<td>0.00-0.12</td>
<td>0.00-0.10</td>
</tr>
<tr>
<td>Base</td>
<td>0.10±0.06</td>
<td>0.10±0.06</td>
<td>0.10±0.06</td>
<td>0.10±0.06</td>
<td>0.10±0.06</td>
<td>0.10±0.06</td>
</tr>
</tbody>
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Figure 1.

Summary/Conclusions: We established a cost-effective 10 color single tube FC-MRD assay with high sensitivity of at least 1 in 10^5 and applicability in >97% BCPALL MRD samples. We also described the frequency and extent of different cellular events and artifacts that can interfere with high-sensitivity BCPALL FC-MRD analysis. The knowledge regarding presence and antigen expression pattern of these cellular events and artifacts are critical to avoid potential false positive results.

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 1). 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 there factors on cardiological parameters could not be evaluated due to low numbers. Echocardiography was performed with Vivid E9 ultrasound system (GE Medical System) and MSRS 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 intermittent controls before each re-exposures (TP150, TP210, TP270). GLS values>19% (or GLS drop >10% from basal value) and LVEF values<65% (or LVEF drop >10% from basal value) were considered as abnormal. Statistical analysis was performed using Student’s t-test.

Table 1.

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Summary/Conclusions: We established a cost-effective 10 color single tube FC-MRD assay with high sensitivity of at least 1 in 10^5 and applicability in >97% BCPALL MRD samples. We also described the frequency and extent of different cellular events and artifacts that can interfere with high-sensitivity BCPALL FC-MRD analysis. The knowledge regarding presence and antigen expression pattern of these cellular events and artifacts are critical to avoid potential false positive results.
with b-blockers, as they could limit anthracycline 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 anthracycline, 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 exon 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 the Illumina® MiSeq® 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, 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 of clonality with specific rearrangements was demonstrated between LymphoTrack® MiSeq and PCR-CE method.

Background: NUDT15 polymorphism has been recently identified as a determinant of thiopurine intolerance. 6-thioguanine nucleotides (6-TGN) is monitored to prevent hematopoietic toxicity in acute lymphoblastic leukemia (ALL). Aims: This study intended to evaluate the impact of NUDT15 polymorphism on thiopurine intolerance and 6-TGN level in Korean children with ALL.

Methods: Genotyping of NUDT15 was performed in 258 children with ALL who were registered in Samsung Medical Center. According to NUDT15 diplotype, patients were classified into low risk (LR, wild-type), intermediate risk (IR, heterozygous variant) or high risk (HR, homozygous or compound heterozygous variant). 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).

Results: The least 6-mercaptopurine (6-MP) dose (mg/m²/day) were administrated at 0.9, 2.6, 5.9, 9.5, 13.8, 15.1, 16.9, 19.0, 21.5, 25.0, 28.7, 31.2, 34.0, 35.9, 38.7, 41.9, 43.5, 45.3, 47.1 mg/m²/day for LR, IR, and HR, respectively. The lowest WBC and platelet counts and hemoglobin level were observed in HR. 6-TGN level (pmol/8x10⁸ RBC) divided by 6-MP dose (mg/m²) was the lowest in HR group (4.4 vs IR 13.3 vs HR 14.7, p<0.01).

Summary/Conclusions: Patients with NUDT15 variants encountered significant thiopurine intolerance even with low level of 6-TGN. This concurs with the existing hypothesis that NUDT15 protein may prevent incorporation of thiopurine active metabolites into DNA. Therefore 6-TGN monitoring is not useful to predict hematopoietic toxicity for patients with NUDT15 variant.

E847 DETECTION OF CLONALITY IN CLINICAL SPECIMENS FROM SUSPECTED B-CELL MALIGNANCIES USING COMPREHENSIVE IGH LYPHOTACK® MISEQ® AND PGAM ASSAYS

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Background: PCR-based capillary electrophoresis (PCR-CE) methods targeting immunoglobulin heavy chain (IGH) were designed to include both platform specific adapter sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGAM platforms. MiSeq IGH FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. PGAM IGH FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. DNA was extracted from 21 PB, 37 FFPE and 1 BM clinical samples. Single step PCR amplification of 50 ng DNA input was followed by ampiclon purification. Equimolar amounts of purified amplicons were pooled and loaded onto the sequencer. MiSeq and PGAM instruments used were the LymphoTrack® IGH (FR1, FR2, FR3) Assays for both the illumina® MiSeq® and Thermofisher Scientific® ion PGM™ platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LymphoTrack® IGH MiSeq and PGAM Assays to the IGH PCR-CE assay by testing in 59 anonymized, blinded clinical samples.

Aims: To assess the clinical performance of LymphoTrack® IGH MiSeq and PGAM Assays

Methods: LymphoTrack® IGH Assay has been developed for both the MiSeq and PGAM platforms. Proprietary consensus primers targeting the V and J gene segments of IGH were designed to include both platform specific adapter sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGAM platforms. MiSeq IGH FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. PGAM IGH FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. DNA was extracted from 21 PB, 37 FFPE and 1 BM clinical samples. Single step PCR amplification of 50 ng DNA input was followed by ampiclon purification. Equimolar amounts of purified amplicons were pooled and loaded onto the sequencer. MiSeq and PGAM instruments used were the LymphoTrack® IGH (FR1, FR2, FR3) Assays for both the illumina® MiSeq® and Thermofisher Scientific® ion PGM™ platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LymphoTrack® IGH MiSeq and PGAM Assays to the IGH PCR-CE assay by testing in 59 anonymized, blinded clinical samples.

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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 transplantation. 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 general 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. This assay was sensitive to as many as 1 x 10^6 cells. 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 coefficient.

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 unquantifiable 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=0.904, 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.
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


Summary/Conclusions:

We examined the association between NUDT15 polymorphism and clinical outcome of children with acute lymphoblastic leukemia (ALL) in Korea. 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.

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 the backbone of ALL treatment regimen. Genetic polymorphism in thiopurine methyltransferase (TPMT) is well known to affect the 6-MP tolerance. However prevalence of non-function variant of TPMT is rare in Far East. Recently, a study has identified a variant of the NUDT15 gene associated with intolerance of 6-MP.

Aims: We examined the association between NUDT15 polymorphism and clinical outcome 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 ration of prescribed 6-MP dose over protocol planned dose.

Results: We found two kinds of variants, c.55_56insGAGTCG(rs869320766) in exon 1 from 8 patients and c.415C>T(rs116855232) in exon 3 from 14 patients of them, 7 patients had both variants and all variants were 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 small 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>Admission day during maintenance (Day)</th>
<th>5-year EFS (%)</th>
<th>5-year OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>26</td>
<td>2 (7.7%)</td>
<td>13 (5.4-25)</td>
<td>98.0±2.5</td>
<td>98.2±1.5</td>
</tr>
<tr>
<td>c.415C&gt;T</td>
<td>8</td>
<td>3 (37.5%)</td>
<td>78.3±45.8</td>
<td>87.5±11.7</td>
<td>100.0</td>
</tr>
<tr>
<td>c.55_56insGAGTCG</td>
<td>8</td>
<td>1 (12.5%)</td>
<td>198.0±33.5</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>c.415C&gt;T, c.55_56insGAGTCG</td>
<td>1(100%)</td>
<td>32</td>
<td>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 background 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 was 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 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), 10-13 years old (76% vs 57%, P<0.0001), and 20-24 year old patients (51% vs 31%, P=0.0116). In univariated 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 TO SIMULATION MODEL

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Background: The role of autologous hematopoietic stem cells transplantation (aHSCT) for patients with T-cell ALL is still being discussed. The resent 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 cannot explain the magnitude of the effect of aHSCT on patient’s survival. The methods: We have developed SAS macros time-depend graphical and analytic procedures for time dependent factors: Land Mark (LM) methods, Mantel-Bay test, Cox regression model (CM) and also a base for simulation all end points and study events like remission, transplantation, relapse and death are well approximated by a mixture of exponent distributions. Non-constant (dropping) hazard rate exists in real study data. The consequence of violation of constant hazard assumption as most possible source of biases was tested on our 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-myeloablative BEAM conditioning was scheduled as late 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 (T/I) phenotype was verified in 56
(52.3%), mature (T-IV) - in 10 (0.4%), thymic (TII, CD1a+) ALL - in 41 pts (36.3%). 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 - $P_{M/B}=0.0004$, Cox model output: 1/HR=15.9, $P=0.008$. Simulation model for remission consists of 3 fractions: early ($\alpha=10\%$, $\tau=0.05\$ m, $\delta=0\$ m), normal ($\alpha=57\%$, $\tau=0.28\$ m, $\delta=1\$ m) and late remission ($\alpha=33\%$, $\tau=1.31\$ m, $\delta=2.2\$ m), for survival consists of 2 fractions: short life ($\alpha=59\%$, $\tau=22\$ m), long life ($\alpha=41\%$, $\tau=600\$ m), (Fig.2). The first simulation experiment was performed in preposition that transplantation has no effect (HR=1). To exclude the random effect the sample size was N=4000, Mantel-Bay and Cox model show significant ($P_{M/B}=0.50$, $P_{Cox}=0.50$, HR=93) but LM plot demonstrates recognizable bias in transplanted patient group (Fig.3). The second experiment supposed that the existed effect of aHSCT (HR=0.5), N=5000. Mantel-Bay and Cox model would show significinance, but hazard rate was underestimated (PMB=.03, PCM=.03, HR=70. (0.50-0.97)). Other experiments were done for repeated simulation, which demonstrated a very good agreement of Mantel-Bay and Cox methods and their robustness.

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 underestimates 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 ALLOGENIC 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 mot 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), Lymphosar Daunorubicine (DNSX), 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 idie until the achievement of CR. FLAD regimen consisted of a three-days administration of Flu 30mg/sqm followed by Ara-C 2000mg/sqm and DNSX 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. Then to cure them we used 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 5/8 patients proceeded to HSCT. Two patients are currently waiting for transplant. Overall, 6 patients are alive and in MRD negative CR at the time of analysis. One patient died at day +289 after SCT due to non-relapse mortality and one has died after the first FLAD in molecular CR because of an unrelated event.

Summary/Conclusions: This therapeutic strategy proved to be very 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 STERoidal 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 cleary showed 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 results with high dose methilprednisolone (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 concomittant 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 treatment 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 8400/mm$^3$ (1100-55300/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 analy-sis. 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 were all 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.
Acute leukemia microenvironment is essential for development of new treatment options. Further knowledge gained from multiple cytokine and adhesion molecule evaluation could help to improve treatment outcomes. Aims: The aim of this study was to evaluate baseline levels of cytokines, cytotoxic receptor molecules 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, specifically 21 cytokines, 4 soluble cytokine receptors, 5 soluble adhesion molecules and 1 hormone (C-peptide). All analytes were measured by biochip array technology on Evidence investigator analyzer (Randox). Serum levels of tested analytes were correlated with baseline characteristics and prognostic factors, such as age, sex, risk group according to GMALL (SR 9, HR 9, VHR 12 patients), full blood count parameters (including percentage of blasts), biochemical parameters (LDH, CRP), response to induction therapy (CR rate after induction), progression-free survival (PFS) and overall survival (OS).

Results: Comparing analytes with baseline characteristics, we found significant negative correlations between IL-7 and leukocyte count (r=-0.633; p=0.032), percentage of blasts in peripheral blood (r=-0.695; p=0.004) and LDH (r=-0.604; p=0.075). Furthermore, we found significant positive correlations between IL-7 and sGLUT4 (r=0.801; p=0.0001), sIL-2R and CD163 (r=0.573; p=0.0012). Correlations with baseline risk stratification according to GMALL did not reach statistical significance. In the study population, CR rate after induction was 86% (MFD negative in 29%), 1-year PFS 68% and 1-year OS 73% (2 patients died during induction therapy). Higher levels of EGF were associated with failure to achieve CR after induction therapy (r=0.689; p=0.073). So far, no significant correlations between baseline analyte levels and inferior PFS or OS were found. In newly diagnosed ALL patients, we found statistically significant correlations between sTNFR-1 and sTNFR-2 (r=0.805; p=0.0001), IL-1α and IL-4 (r=0.700; p=0.008), sTNFR-2 and M-CSF (r=0.657; p=0.037), sTNFR-2 and VCAM-1 (r=0.652; p=0.004). Correlations between IL-7 and other analytes did not reach statistical significance.

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 in resistant disease. This finding is similar to 61.2% of JL1 expression in adult ALL 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 E. You1, S. Kim2, C.-J. Park1, S. Jang1, Y.-U. Cho1, C.H. Yoon1, K.-N. Koh3, H.J. Im1, J.J. Seo2

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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 antigen was measured using the JL1 monoclonal antibody (Beckton-Dickinson, San Jose, 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: 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 expressions than negative JL1 group in CD 14 (P=0.043), CD7 (P=0.026), CD56 (P=0.016) and lower expressions in CD65 (P=0.05). With regard to ALL patients, CD 20 (P=0.002) and CD2 (P=0.005) expressions were significantly higher in JL1 positive group than JL1 negative group. Positivity of JL1 antibody did not show significant difference between B- and 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 demonstrate that anti-JL1 antibody might be used in childhood acute leukemia patient showing JL1 expression.

E858

SERUM LEVELS OF CYTOKINES AND ADHESION MOLECULES AND THEIR ASSOCIATION WITH PROGNOSTIC FACTORS IN NEWLY DIAGNOSED ACUTE LYMPHOCYTIC LEUKEMIA PATIENTS M. Moreno1,2,3, K. Kusagawa1, K. Okuda1, L. Favaro1, P. Vassough1, P. Sasaki1,2,*, T. Kussa1,2, J.M. Horacek1,2,*, T. Vanek3, L. Jebavy1,2, P. Zak2

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Background: Dysregulated production of cytokines and adhesion molecules has been implicated in the onset and progression of various types of leukemia. Further knowledge gained from multiple cytokine and adhesion molecule evaluation could help to improve treatment outcomes.

Aims: The aim of this study was to evaluate baseline levels of cytokines, cytotoxic receptor molecules 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, specifically 21 cytokines, 4 soluble cytokine receptors, 5 soluble adhesion molecules and 1 hormone (C-peptide). All analytes were measured by biochip array technology on Evidence investigator analyzer (Randox). Serum levels of tested analytes were correlated with baseline characteristics and prognostic factors.

Results: Serum levels of cytokines and adhesion molecules have been implicated in the onset and progression of various types of leukemia. Further knowledge gained from multiple cytokine and adhesion molecule evaluation could help to improve treatment outcomes.
Background: The survival of 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 of 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 patient’s 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 phase 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. Outcomes of OS and DSS were not significantly different between the two groups. Incidences of Neutrophil engraftment and acute GVHD incidence were not statistically different between IMA-pts and DAS-pts. Incidence of chronic GVHD was marginally increased in IMA-pts (IMA; 63%, DAS; 33%, P=0.06). At the median follow-up of 10 years (range: 14-460 days), overall survival was not different between the two groups by univariate analysis (Logrank, P=0.16). However, by multivariate analysis using Cox regression model for adjusting confounding factors, including, overall survival was superior for IMA-pts [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 (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 Imatinib. 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: NCT00793933) 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 MoCR on day 70 of treatment. MoCR 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: MoCR on day 70 was achieved in 36% and 59% in RALL–2009 (n=44) 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 MoCR 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 (RALL–2009 and RALL–2012) was similar: OS – 62.8% vs 49.4% (p=0.6), RFS – 55.7% vs 45% (p=0.7), respectively. In order to evaluate the impact of allogeneic HSCT we performed a comparison of transplanted and non-transplanted patients by a landmark analysis. The landmark was chosen as the median time from CR to allo–HSCT – 4,3 mo (3-16 mo). So, the 5y OS from allo–HSCT was 65.6% in transplanted patients (p=0.18), and RFS was 25% vs 62.5% (p=0.19), respectively. OS for older pts (>45 y) was 40% vs 25% in transplanted vs non-transplanted group of the pts, and RFS was 25% vs 66.6%, respectively. OS in younger (≤45 y) pts was 83.3% vs 58.9% for transplanted vs non-transplanted pts, EFS was 77.1 vs 21.4%, respectively.

Summary/Conclusions: The results very pessimistic in older (>45 years) patients who received HSCT. The contrary was observed in younger adult patients with very good results after HSCT – OS was 83.3% and EFS 77.1%. We conclude that patients aged>45y should continue chemotherapy without allogeneic HSCT or may be we could apply autologous HSCT for that group of the patients.

E861
TARGETABLE BLINATUMOMAB + TYROSINE KINASE INHIBITORS TREATMENT IN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS: CLINICAL EFFECTIVENESS AND PERIPHERAL LYMPHOCYTES SUBPOPULATIONS KINETICS
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Background: Blinatumomab is a bispecific monoclonal anti-CD3/CD19 antibody which has clinical activity in relapsed/refractory Ph-positive acute lymphoblastic leukemia (ALL) as monotherapy. Combination of Blinatumomab with...
tyrosine kinase inhibitors (TKI) is the promising approach in treating Ph-positive ALL. Some other rearrangements like IKZF1 in Ph-like ALL, FLT3 and JAK2 in Ph-negative ALL are the potential targets to some TKIs.

**Aims:** To demonstrate effectiveness and toxicity profile of Blinatumomab+TKI treatment. To evaluate peripheral blood lymphocytes subpopulations kinetics during blinatumomab treatment.

**Methods:** From October 2015 to February 2017 10 patients (pts) aged from 24 to 42 (median 31), 7 female and 3 male, with relapsed/refractory ALL were treated in our center. The diagnosis was relapsed ALL in 8 pts (7 - overt hematological, 1 - cytogenetic relapse) and persistent/increasing minimal residual disease of ALL in 2 pts. All pts had strong CD19 positivity. 8 pts was diagnosed as Ph-positive ALL (p190), 1 - Ph-like ALL (IKZF1 rearranged). 1- FLT3+ ALL. Two pts has T315I ABL mutation. In all pts blinatumomab continuous infusion + TKI therapy was started. Blinatumomab dose during 1st week of 1st cycle was 9 mcg/day, 28 mcg - subsequent three weeks. Blinatumomab dose in subsequent 4-weeks cycles was 28 mcg/day. 7 pts were treated with TKI Dasatinib, 1 - Bosutinib (Dasatinib/Nilotinib interloater), 1 - Ponatinib (T315I), 1 - Sorafenib (FLT3+). ATRA was added to Dasatinib in 1 pt with IKZF1 rearranged Ph-like ALL. 1 pt received 1 cycle of 4 weeks blinatumomab, 1 pt – 2 cycles, 6 pts - 4 cycles, 2 pts – 5 cycles. TKIs were administered continuously in all pts. T-helper, T cytotoxic, T-regulatory and NK cells were measured by flow cytometry in every week during all cycles of blinatumomab treatment.

**Results:** No one pt has neurological toxicity of any grade. All pts has significant decrease of normal Ig level and all of them received intravenous human normal immunoglobulin replacement. Palmar-planter syndrome in one pt on sorafenib completely resolved after temporarily TKI discontinuation. Disappearance in 1 pt on dasatinib/nilotinib completely resolved on bosutinib. 8 pts achieved molecular remission (MoCR), one pt – cytogenetic remission and one pt with T315I progressed to overt hematological relapse. T-helper and T-lymphocyte subpopulations were on or below of lower limit of normal range. T-cytotoxic and NK subpopulations gradually returned into normal range (Fig. 1). AlloBMT was performed in 4 pts. Three pts are awaiting alloBMT and three are continuing Blinatumomab + TKI treatment.

**Summary/Conclusions:** Lowering toxicity in non-chemotherapy treatment has its significance in such a heavily pretreated patients with relapsed ALL. The treatment has high MoCR rate and low toxicity profile. Treatment effectiveness correlated with T-helper and T-regulatory subpopulations 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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**Background:** Cytokine receptor-like factor 2 (CRLF2) plays an important role in the development of normal B lymphocytes, which can mediate early B cell proliferation and survival. CRLF2 overexpression and rearrangement have been observed in acute lymphoblastic leukemia (ALL), and they are reported to contribute to oncogenesis and unfavorable outcome in ALL. We reported that CRLF2 overexpression in the patients without CRLF2 rearrangement, indicating the reason other than CRLF2 rearrangement is responsible to the CRLF2 overexpression. There is few reported CRLF2 mutations in adult ALL.

**Aims:** We aim to investigate the mutations of CRLF2 and its clinical significance in adult ALL without CRLF2 rearrangement.

**Methods:** The 129 patients’ BM samples (95 B-ALL, 33 T-ALL and 17 B-T-ALL) were collected between April 2010 and Jan 2015 at the First Affiliated Hospital of Nanjing Medical University. The ALL diagnosis was made according to the cytogenetic, morphologic, Immunophenotypic and molecular criteria. The mutations of CRLF2 were analyzed by MiSeq (Illumina) and Sanger sequencing.

**Results:** A total of 7 mutations were identified in 129 patients without CRLF2 rearrangement. Six were single nucleotide mutations (Ser394Leu, Thr395Met, Ser414Asp, Ser421Asp, Asp422Glu and Ser422Gly) and one was a small insertion (Ser422Ile). The most frequent mutations were Ser414Asp and Ser422Gly. The Ser414Asp and Ser422Gly mutations were observed in 32 (24.6%) and 21 (16.2%) patients, respectively. The Ser422Gly mutation was the most common mutation in patients without CRLF2 rearrangement.

**Summary/Conclusions:** The rate of 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.

**E862**

**VERY VERY LATE RELAPSES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE SERIES**

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**Background:** Recurrence of acute lymphoblastic leukemia (ALL) during childhood usually occurs within the first six years after initial diagnosis.

**Aims:** The aim of this study is the identification of all relevant characteristics and outcomes in a group of patients with childhood ALL, who relapsed more than six years after initial diagnosis or more than three years after bone marrow transplantation (BMT). We aimed to determine the incidence of all relevant characteristics and outcomes in a group of patients with childhood ALL, who relapsed more than six years after initial diagnosis or more than three years after bone marrow transplantation (BMT).

**Methods:** All children diagnosed with a first relapse of ALL in our Department, from January 1992 till December 2010 were included in this study.

**Results:** During this period, a total 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, 107, 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.XY,t(19;22)(q11.2;q12) (t(12p13q14?); none hadMLL rearrangement orETV6-RUNX1. In 9 cases, the cytogenetic profile remained identical at recurrence, while in 1, trisomia 13 was not detected and another had heterozygous absence of IKZF1, PAIX, EBF1, CDKN2A and CDKN2B genes. On Day 8, nine of 11 patients were Prednisone Good Responders. On Day 15, nine children had bone marrow control, one M1 and two 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, 11 and 20 years after initial diagnosis. One patient had died from second recurrence and the last two had a second allogeneic BMT and died due to severe infection, 2 and 11 months following that 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 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 (8.53×109/L vs 28.9×109/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 (248.4×109/L) and low platelet count (10×109/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 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 KITAS (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 CCND2 (9/95; 9%), that plays an important role in regulation of hematopoietic cell proliferation, as well as DHX15 (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 leukemias 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 leukemic blasts from 3 AML (MLL+) patients. Signalling events were evaluated by immunoblotting, p65 mediated BTK expression was determined by promoter assays. Cells were treated with specific inhibitors of BTK (Ibrutinib (IBR): 0.25, 0.5, 1.0 and 2µM) in combination with Daunorubicin (DAU 5nM) or RAC (NSC 23766 (NSC): 5, 10, 15 and 20µM) for 48 hrs and cell viability was assessed using Annexin V Sytox Blue based flow cytometric analysis.

Results: FLT3-ITD driven AML is 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 7.6) and leukemic blasts from the 3 AML patients. Active BTK (pY223) was detectable in both the clones of MA9 and MLL+AML samples. Interestingly, the cells demonstrated activation of p65 (p53S6) but not in control cells. To address if activated p65 could potentially drive BTK expression, we performed BTK promoter assays with reporter construct and empty vector. MA9.3 cells electroporated with test construct demonstrated significantly higher transcriptional activity. At the protein level, p65 inhibitor treatment (MG132 or Bay 11-7082) reduced total BTK expression, indicating that p65 stabilized a related p65 mediated creation of BTK-6. 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 (CI) values indicated that IBR-DAU combination synergistically reduced cell viability (CI > 1.0-antagonistic; <1.0 synergistic and =1 additive effect). Recent studies suggested RAC-GTPase signaling may also be a target in AML, particularly in the context of FLT3-ITD. Similar experiments were performed in FLT3-ITD driven AML cell lines and empty vector. MA9.3 cells electroporated with test construct demonstrated significantly higher transcriptional activity. At the protein level, p65 inhibitor treatment (MG132 or Bay 11-7082) reduced total BTK expression, indicating that p65 stabilized a related p65 mediated creation of BTK-6. 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).

Summary/Conclusions: Taken together, our data support a biological link between NFκB, BTK and RAC pathways in the modulation of cell survival in MLL-rearranged AML cells. aberrantly active p65 drives the expression of BTK and contributes to the progression of the AML. Combination treatment using IBR-DAU and IBR-NSC might be a promising therapeutic strategy, minimizing high drug dose-related side effects but increasing the therapeutic efficacy.

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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 CD33/CD3 BiTE® antibody construct (AMG 330) is able to recruit autologous, residual T cells and induce cytotoxicity against primary AML cell lines. However, as described previously (Musaii et al, Blood 2013) primary AML cells are able to secrete soluble factors, which might not only influence T-cell proliferation but also negatively impact 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 from bone marrow (BM) 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 vs FCS: 78.6 vs 95.0, respectively). In contrast, AMG 330 mediated T-cell proliferation was increased in 14/30 experiments (mean% specific proliferation vs FCS: 78.6 vs 95.0, respectively).

Conclusion: Our results demonstrated long term culture system (Krupka et.al, Leukemia 2016) and HD T cell lines. In flow cytometry based experiments we determined the influence of AML plasma from bone marrow (BM) of AML patients 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.

E868

A PRECISION MEDICINE PLATFORM FOR ACUTE MYELOID LEUKEMIA TO HELP UNRAVELING THE MOLECULAR ADDICTIONS OF FLT3-ITD-DRIVEN AML

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Background: Acute myeloid leukemia (AML) is an aggressive disease with poor prognosis (Tzelepis et al., 2016). No single driver mutation is present in all cases of AML, making its treatment a challenge (The Cancer Genome Atlas Research Network, 2013). Traditional standard of care for AML is an aggressive induction therapy aimed to uncover driver mutations that have remained unchanged for the past 30 years (Longo et al., 2015). Weak or elderly patients might not be eligible for intensive treatment, leading to poor survival rates. Many such patients are labeled as ‘untreatable’, although a portion of them could benefit from specific, individualized treatments. The use of Precision Medicine strategy can help to find the specific treatment for these ‘untreatable’ AML patients.

Aims: Drug-driven personalized medicine aims to directly test the sensitivity of primary cancer cells taken from individual AML patients to a selection of targeted cancer drugs, compare these results with drug sensitivities of healthy donor samples and select the most effective drug for each patient. This approach considers any combination of mutations or epigenetic changes that might not be found in the standard sequencing panels, an advantage when dealing with such a heterogeneous disease. Proof of principle of this strategy was recently demonstrated by FiMM (Helsinki, Finland) (Pernovska et al., 2013), not only providing immediate clinical benefit to leukemia patients, but also identifying drugs that can potentially be repurposed for future treatment of patients.

Methods: We have established a drug-driven personalized medicine platform for AML where we check the ex-vivo drug sensitivity and resistance of bone marrow primary cells to a panel of around 400 drugs and drug combinations covering the standard of care treatments, cancer chemotherapeutics as well as many clinically available and emerging molecularly targeted compounds. We calculate the IC50 values for all the drugs for each individual donor or patient, and then the differential drug sensitivity scores, selecting the drugs that affect preferentially the cancer cells when compared with healthy cells. To date we have successfully processed 6 healthy donors and 68 AML patients, identifying subgroups of patients who respond with a similar dynamic to certain classes of drugs, as the subgroup of cells carrying intrinsic tandem duplications in the receptor tyrosine kinase FLT3 (FLT3-ITD).

Results: FLT3 activating mutations, particularly FLT3-ITD, have been observed in approximately 30% of AML cases (The Cancer Genome Atlas Research 2013), and are associated with increased risk of relapse and poor clinical outcome (Abu-Duhier et al., 2008). The cellular pathways that support FLT3-ITD-expressing AML blasts show an enhanced sensitivity to HSP90 inhibitors such as Ganetespib compared to healthy donors and any other subgroups of leukemia. In addition, HSP90 inhibitors specifically sensitize FLT3-ITD expressing bone marrow-derived cells to TKIs, whereas cells derived from healthy donors are unaffected. HSP90 inhibitors also preferentially eradiate a population of patient-derived FLT3-ITD+ AML cells expressing leucemic stem cell markers.

Summary/Conclusions: In summary, our study reveals a molecular basis for HSP90 addiction of FLT3-ITD-driven AML and provides a rationale for treatment of this form of AML with HSP90 inhibitors.

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Summary/Conclusions: In summary we demonstrated that BM derived plas-
tic cells are sensitive to stromal conditioned medium. Stromal secreted 
proteins mediate AML resistance. In vivo experiments confirmed a role 
for these proteins in AML resistance. These findings suggest that tar-
ted inhibition of stromal proteins may increase the effectiveness of 
existing and novel treatments in AML.

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 dependent on stromal conditioned media to survive long-term in culture. Although some of the components of the stromal secreto
me (the totality of secreted proteins by biological cells) that augmented AML survival are known, the precise molecular mechanisms of the stromal-blast interactions are not fully defined.

Aims: To study the clonal evolution of FLT3-ITD-AML and FLT3-TKD-AML in vivo and ex vivo and to investigate the influence of the stromal microenvironment on AML blast protein expression and AML cell signaling.

Methods: We used primary AML cells and established cell lines. Four different human AML cell lines were grown 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 stromocyte (in triplicate). Proteins from these secreto
mes 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 prote
ins. Guava EasyCyte Flow Cytometry was used to measure the viability and proliferation of these cell populations, assessing the capabilities of the identified factors above on primary AML cells (n=6) as well as the effects of kinase inhibitors midostaurin and dasatinib on AML cell viability.

Results: We identified 473 secreted proteins that significantly increased AML cell viability/ proliferation. The most prominent factors (top 20) included TGFBL, LIF, IL6, IL8, and GRO. Using a combination of stromal lines and conditioned media, we observed a significant increase in AML cell proliferation and survival.

Conclusions: Our findings suggest that the microenvironment secreted proteins play a critical role in AML cell survival and proliferation. Further studies are needed to determine the specific mechanisms by which these proteins contribute to AML cell resistance to targeted therapy and to identify potential therapeutic targets.
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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. 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 theFLT3mut status at the time of diagnosis (Dx), refractory disease (RD) and relapse (Rel) in AML pts treated within the CALGB 10603 RATIFY trial and is still under investigation within the AMLSG 16-10 trial; one pt had an additional FLT3-ITDmut. Pts entered in the RATIFY trial had either FLT3-ITD (n=22), a FLT3-TKDmut (n=9), or both (n=2). The median AR of FLT3-TKDmut at Dx was 0.82 (0.07-2.66) and the majority of pts showed loss of FLT3-TKDmutat 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 fraction of the FLT3-ITD mutations 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, FLT3ITD 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 [0.65 (0.08-9.1); p<0.002]. 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 presence of ITD clones that have the potential to mediate 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.

E871

A NOVEL PML-RARA FUSION IN ACUTE PROMYELOCYTIC LEUKEMIA

Background: Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) characterized by specific translocation involving retinoic acid receptor alpha (RARA) locus. Retinoic acid receptor (RAR) is a member of nuclear receptor family, and has three types of isoforms such as RARA, retinoic acid receptor beta (RARB) and retinoic acid receptor gamma (RARG). Both RARalpha and RARbeta has a high structural homology (90%). However, RARALPHA and RARBelta have different oncogenic properties of the artificial PML-RARF fusion gene was observed in an in vitro study, there has been no report on the PML-RARF fusion in human APL patients.

Aims: We report here a novel PML-RARF rearrangement in a patient with AML displaying the suitable morphologic and immunophenotypic features of the classic hypergranular APL.

Methods: Whole genome sequencing (WGS) and further analysis of mRNA and gDNA were performed to clarify the atypical gene rearrangement observed by karyotyping and FISH.

Results: Laboratory and immunophenotypic analysis results suggested the classic APL with hypergranular type. A clonal translocation t(12;15)(q13;q22) was identified by karyotyping. No evidence of fusion of PML-RARA was detected by RT-PCR and PML-split was found on FISH analysis using PML-RARA dual color dual fusion probes. WGS analysis performed to clarifying the partner gene of PML located on chromosome 12q13 strongly suggested a PML-RARF fusion. RT-PCR following sanger sequencing were performed to verify the presence of PML-RARF fusion transcript, then two kind of transcripts was detected, one with the fusion of PML exon 3 and the middle part of exon 1 of RARG and the other with the fusion of PML exon 3 and exon 2 of RARG. The breakpoint of DNA was clarified on intron 3 of PML and 5′ region of RARG. Despite of ATRA treatment for 9 days, cell count did not show any response. Then induction chemotherapy composed of idarubicin and cytarabine was combined on ATRA. ATRA was finally stopped after 18 days, then cytokrogenic remission was achieved day 36 after induction therapy.

Summary/Conclusions: We first report the presence of PML-RARF fusion in a human APL patient. This report supports the possibility of a new molecular mechanism involving RARG not RARA in APL and suggests the need of different therapeutic approach for this variant case showing the potential ATRA resistance.
bone marrow microenvironment (BMM). Survival of patients with AML is presently poor: two-thirds of younger adults, and 90% of older adults die of their disease. Even in patients who achieve remission with chemotherapy, relapse is common and occurs from minimal residual disease sequestered in protective niches in the BMM. Reciprocal interactions between that of the AML and bone marrow mesenchymal stromal cells (BM-MSC) are central to the survival and progression of blasts that are protected from the hypoxic microenvironment. Restoration of quiescence in malignant cells as well as the activation of anti-apoptotic and pro-survival pathways.

Aims: To investigate how BM-MSC are programmed by AML to generate a pro-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 MicroBead selection. AML blasts co-cultured in 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 supported the findings carried out to compare 10 healthy and 10 AML BM-MSC show that 1125 genes were differentially expressed, with 924 down-regulated in AML derived BM-MSC and 201 up-regulated. From this analysis, we found that CDKN1A (p21) is up-regulated in BM-MSC from AML patients (7.406 logFC) compared to BM-MSC from patients with normal bone marrow (0.232 logFC). p21 mRNA and protein expression is increased in 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 resulted in increased proliferation and survival. 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.

E875

BONE MARROW ECLOGICAL COLAPSE IN ACUTE MYELOID LEUKEMIA IS MEDIATED BY REMODELING OF ENDOSTEAL VESSELS

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Background: Bone marrow vascular niches have been proposed to support acute myeloid leukemia (AML) growth. However, anti-angiogenic therapies do not improve patient outcome suggesting that a complex relationship between AML cells and the microenvironment influences the disease process.

Aims: We aim to study the complex vascular remodelling occurring during AML progression.

Methods: Using a murine model of AML we performed intravital microscopy to study the remodeling of endosteal vessels in the bone marrow.

Results: We show AML is an invasive species causing highly localized disruption of the endosteal stroma and outcompeting non-malignant cells. Particularly affected are endosteal microenvironments containing osteoblastic cells and type H endothelium, typically associated with hematopoietic stem cells (HSCs). Infiltration of AML expands osteoblastic and HSC niches, suggesting their capacity to support extramedullary hematopoiesis. Intravital microscopy further revealed that the endothelium in AML is more adhesive and permissive to transendothelial migration of hematopoietic cells. Pharmacological intervention known to induce type H endothelium preserved HSC niches in suboptimal areas.

Summary/Conclusions: Together, these data suggest that AML-induced vascular damage contributes to cell egress from the bone marrow, and that new therapeutic approaches aiming to normalize bone marrow vasculature may support normal hematopoiesis.
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 xenotransplantations. Similarly, eight PDX with respective primary AML were analysed by next-generation sequencing (NGS) of 27 AML relevant genes. We found stable variant allele frequencies (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 CD3ζ chain of the T-cell receptor (TCR) complex. Recent studies have shown that the mechanism of 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/CD3 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 intravitro cocultures, cytokoticticy 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 cytototoxicity 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 median fluorescence intensity (MFI) ratio: B33 116.1 vs MOLM-13 67.6). However, through the addition of an anti-CD28 antibody or recombinant human IL-2, cytokoticticy against B33 cells could be restored (% specific lysis AMG 330 vs AMG 330 + anti-CD28 vs AMG 330 + IL-2: 58.3±32.9 vs 82.6±11.1 vs 91.3±9.0). At lower E:T ratios (1:4) the additional costimulatory signal also increased AMG 330 mediated cytototoxicity against MOLM-13 cells (mean MFI Ratio of CD80 and CD86 on MOLM-13 cells: 1.4±1.2 and 3.0±1.0 respectively, n=3) (% specific lysis AMG 330 vs AMG 330 + 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. Anti-CD3/anti-CD28 antibodies served as positive control. In the presence of target cells, AMG 330 induced significantly lower Akt and Erk phosphorylation (mean% phosphorylated (p)Akt and pErk 7.9 and 7.6, n=3) compared to crosslinked CD3/CD28 antibodies (mean% pAkt and pErk 43.0 and 34.6). However, the combination of AMG 330 and CD28 increased the amount of phosphorylated proteins (% mean pAkt and pErk 11.6 and 11.1), but not to the level achieved by CD3/CD28 stimulation. In the absence of target cells, no Akt phosphorylation was observed upon incubation with AMG 330, suggesting a highly target cell dependent T-cell activation (mean% pAkt with vs without target cells: 0.8 vs 7.9).

Summary: 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 distinct TCR components. Our results will contribute to the understanding of BiTE® mediated activation of T cells, which is a prerequisite for clinical responses.

E879 RAKF 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 leukaemia (AML), where leukemic invades 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 RAKF 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 only by histology were carried out by exome sequencing and targeted Next Generation Sequencing (NGS). For functional assays, both RKIP overexpression and knockdown was performed in THP-1 AML cells by lentiviral transduction of a FLAG-tagged RKIP expression construct and by RKIP shRNA, respectively. Subsequently, these cells were tested in migration and invasion assays using transwell methodology.

Results: This study comprised 14 patients with MS (MS-group) and 14 patients with AML without any evidence of extramedullary involvement (BM-AML group). Of the 14 cases within the MS-group, MS occurred as isolated event in three cases and concomitantly with systemic AML in eleven cases. Both groups were analysed 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=0.0339). Interestingly, RKIP loss in MS specimens of cases with concomitant systemic AML was also present in the corresponding leukemic BM samples, thereby excluding a geographical clonal heterogeneity during MS formation in respect to RKIP expression. We then analyzed RKIP mRNA levels by qPCR and observed that RKIP loss correlated with decreased expression of RKIP protein (n=14, 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 double overexpression and knockdown of RKIP in the RAS-mutated THP-1 AML cell line and subsequently studied these cells in functional migration and invasion assays. Importantly, RKIP knockdown increased both migration and invasion, thereby indicating a role of RKIP in the development of this condition.

E880

INHIBITING MIR-10A OVERCOMES CYTARABINE-RESISTANCE IN ACUTE MYELOID LEUKAEMIA
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Background: Chemoresistance is the principle cause of treatment failure in acute myeloid leukaemia (AML) despite a promising response to induction chemotherapy. Emerging evidence suggests the roles of autophagy, a self-eating process contributing to chemoresistance of leukaemia cells. We previously demonstrated that miR-10a, highly expressed in a subgroup of AML 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 LC3I/LC3II proteins, autophagy-related proteins via Western Blotting and monodansyl-cavaradine (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 Transcript Factor 2 (TGF) gene (TFF2C). The inhibition of either miR-10a itself or CDKN1A by siRNA treatment inhibited autophagy induced by serum starvation, treatment with autophagy inducer, MG132 or p53 stabiliser, Nutlin3a.

Summary/Conclusions: The data suggests miR-10a as an important regulator of autophagy and modulator of the p53-p21 tumour suppressor signaling axis in subtypes of AML. It also emphasizes the significance of autophagy in chemoresistance in AML, supporting the targeting of the autophagy pathway as a potential therapeutic approach for AML.

E881

BY AN MCL-1-DEPENDENT MECHANISM, ALVOCIDIB POTENTIATES THE ACTIVITY OF CYTARABINE AND MITOXANTRONE WHEN ADDED 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 leukemia (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-sequential 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 (e.g., MCL-1) mediated by alvocidib’s CDK9 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 CellTitre 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 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 MVA-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 dependency 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 AML 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 multivariative 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: 178/311) and myeloid differentiation (gene group 2: 178/311) were considered MSI +. Paired DNAs were separately screened Outlier samples with elevated allele counts were flagged for each locus. Based on the MSs (alleles) each AML sample had was calculated for each of the 18 MS loci. we previously developed (Walker et al, Hum Mutat 2016;37:1004). Briefly, 18 microsatellite loci, called 18 MS loci, were sequenced for 80 genes using amplicon-based next-generation sequencing (NGS). Variants were detected by MuTect and Varscan with variant allele frac- tion. A subset of AML and MDS has been found to have RARs pathway activation characterized by a large enhancer at the RARA locus (RARA-high) and/or upregulation of IKBK, a TF associated with RAS signaling, forming the basis of the SY-1425 sensitive tumor identification. Aims: We sought to understand how SY-1425 agonism of RARa acts to promote maturation and halt proliferation of AML blasts locked into an immature cell state by the cancer circuitry. 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 RARa, and ATAC-seq with or without SY-1425 treat- ment. 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 ROS and JUNB, or IKBK, 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 INP and retinoic acid signal- ing 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 com- positions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocytic, macrophage, dendritic, and granulocytic cell types. While APL has a monocytic and granulocytic differentiation, we found that RARa/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 consis- tent with the observed epigenomic alterations including CD11b, CD11c, CD68, and CD38 upregulation. We integrated epigenomic data, DNA accessibility, and SY-1425 response to understand RARa agonist perturbation to cell circuitry.

Enhancer elements directly bound by RARa were associated with greater CR% after induction in univariate and multivariate analyses, to support the differentiation mechanism of action and offering the potential for early biologically relevant data to inform current and future clinical studies.

Summary/Conclusions: A subgroup of the patient samples was defined by a SE driving RARA which co-occurred with SEs driving ROS and JUNB, or IKBK, 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 INP 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 monocytic, macrophage, dendritic, and granulocytic cell types. While APL has a monocytic and granulocytic differentiation, we found that RARa/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 the observed epigenomic alterations including CD11b, CD11c, CD68, and CD38 upregulation. We integrated epigenomic data, DNA accessibility, and SY-1425 response to understand RARa agonist perturbation to cell circuitry.

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AND APOPTOSIS IN ACUTE MYELOID LEUKEMIA (AML)

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 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 had 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 CEBPA and bi CEBPA pts with a cohort of 34 CN AML patients (pts). bi CEBPA pts had a significantly higher percentage of mutations of 5% were significantly associated with one or more groups. We confirmed the mutual exclusiveness of bi CEBPA and NPM1 and the association between GATA2 and bi CEBPA (35.4%). TET2 was frequently mutated in both mo- (43.8%) and bi CEBPA pts (41.7%), but not in wt CEBPA (16.3%). mo- vs wt CEBPA p<.001; bi- vs wt CEBPA p=.004. Mutations in TKD1 or TKD2 of FLT3 were frequently identified in mo CEBPA (25%) and wt CEBPA (17.8%) but not in bi CEBPA pts (2.1%). The FLT3-TKD1/2 mutation frequency in bi CEBPA significantly differs from mo CEBPA (p=0.002) and wt CEBPA (p=0.004). There was a significant difference in the frequency of FLT3-TKD in bi CEBPA (20.8%) vs wt CEBPA (0%) (p<.001). However, mutations in bi CEBPA pts (43.8%) and wt CEBPA (42.6%) were not significantly different. IDH2 was found mutated only in wt CEBPA (21.6%) and mo CEBPA (18.8%). In 48.8% 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 wt CEBPA (0.35%; p<0.001) or mo CEBPA (3.1%; ns). STAG2 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 (log-rank test: p=.01; 95% CI: 1.03-3.91). Only bi CEBPA pts' pTET2 was significantly worse in bi CEBPA pts with a TET2 mutation, but relapse free survival (RFS) and cumulative incidence of relapse (CIR) was not different depending on TET2 mutational status. Mo CEBPA pts also evaluated the clinical impact of GATA2 mutations. For 30 of 48 bi CEBPA pts survival data was available, 15 of these patients showed 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 TET2mut 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.

E887 MUTATIONAL PROFILE OF RELAPSE-RISK GROUPS IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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Background: Although the fusion oncogene PML-RARA is known to initiate acute promyelocytic leukemia (APL), other cooperating mutations have also been implicated in the disease pathogenesis. However, the spectrum of mutations of APL patients within the relapse-risk groups, based on patient leukocyte and platelet counts at diagnosis, are not yet known.

Aims: 1) To identify genetic alterations that might cooperate with PML-RARA in the leukemogenic process within the three APL relapse-risk groups. 2) To find mutations at diagnosis responsible for poor outcome by comparing patients who experiment relapse vs. patients who do not relapse in each group.

Methods: We performed next generation sequencing (NGS) on bone marrow samples of 91 patients diagnosed with APL (PETHMA LPA09/2005/2012) with a median follow-up of 2.8 years (range 0.2-10) (Table 1). APL patients were classified into relapse-risk groups according to initial leukocyte (WBC) and platelet counts (Score Sanz et al. 2000). Libraries were prepared using the TruSight Myeloid sequencing panel. The gene set analyzed included 54 myeloid related genes. Paired-end sequencing runs were performed on a MiSeq (Illumina) genome sequencer. Minimum depth for reliable analysis was fixed at 100x and minimum variant allele frequency (VAF) in 5%. FLT3-TIT mutations were analyzed by fluorescent PCR and capillary electrophoresis. Sequences obtained were analyzed with the Variant Studio v2.1 software (illumina) and the Integrative Genome Viewer (Genome Browser).

Results: Distribution of 91 patients in the 3 relapse-risk groups was: 28 in low-risk group (31%), 48 in intermediate-risk (53%) and 15 in high-risk (16%). We
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 835. Seven patients assigned to intermediate-risk relapsed (7/38, 18%). All but one carried mutations that have been reported as unfavorable in AML (FLT3, PTEN, ASXL1, CUX1 and WT1). By contrast, patients who remain in complete remission in this group, lack mutations with a greater frequency (12/31, 39%). Finally, within the low-risk group 3 patients suffered relapse (3/27, 11.5%) and all of them presented missense mutations in the Ras domain of NRAS at diagnosis (p.Qer65Arg & 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.

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Summary/Conclusions: In summary, the present study shows that the mutational status of NRAS and FLT3 genes could be used as genetic markers for prognosis in APL, especially in the intermediate and low-risk groups, allowing no differences in the mean number of mutations per patient in each risk APL group (p=0.05). Patients who lack mutations belonged to the intermediate (13/48, 27%) and low risk (4/28,14%) groups, except for only one patient (1/15, 6%) in high-risk group. FLT3 was the most frequently affected gene in high risk APL subgroup (10 out of 15); 8 patients carried an FLT3-ITD mutation and 2 patients had amino acid substitutions at codon 835. Seven patients assigned to intermediate-risk relapsed (7/38, 18%). All but one carried mutations that have been reported as unfavorable in AML (FLT3, PTEN, ASXL1, CUX1 and WT1). By contrast, patients who remain in complete remission in this group, lack mutations with a greater frequency (12/31, 39%). Finally, within the low-risk group 3 patients suffered relapse (3/27, 11.5%) and all of them presented missense mutations in the Ras domain of NRAS at diagnosis (p.Qer65Arg & 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).

E888

ANALYSIS OF THE PD-1/PD-L1 AXIS POINTS TO ASSOCIATION OF UNFAVORABLE RECURRENT MUTATIONS WITH PD-L1 EXPRESSION IN AML.

K. Giannopoulos1,2,*, M. Zajac1, J. Zaleska1, A. Dolnik3, A. Siwiec4, O. Jankowska-change in post-remission therapy. for new mutations required for progression in APL, in order to benefit from a

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E888

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 diversification provided by the often complex landscape, resulting in intra-tumor growth. Such data suggest that each cancer is an extremely heterogeneous 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 therapeutics.

Aims: We aimed to understand how sub-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
second consolidation), and sequenced with high-throughput approach. We and detected at diagnosis and at follow-up (after induction, first consolidation or follow-up: a specific region of the four most frequent alterations at diagnosis (Samples at diagnosis sample; From the 32 genes, we use specific primers to amplify the rent Proton System-Thermo Fisher.

Methods: We developed a custom-targeted sequencing panel of 32 genes at diagnosis. All patients had achieve CR at the moment of MRD assessment.

**Results:**

- Only treatment regimens containing DOX caused marked decreases in HEL cell numbers and barcode architectures diverging strongly from the non-treated control cultures. Replicate AML cultures regrowing after treatment with DOX all converged to a very similar barcode architecture, reflecting that relapse following this mono-therapy was driven by predetermined single-cell lineages. Combination of DOX with CYT increased the degree of overall cell elimination by ~10-fold, while addition of DCT to either chemotherapy regimen had little impact (i.e. yielded similar cell number and re-growth kinetics).
- Interestingly, DCT additions nevertheless qualitatively changed which sub-lineages that regrew - specifically making replications more divergent from each other, indicating a more stochastic selection of the cells emerging when DCT had been added to the respective chemotherapy regimens. Importantly, this cell selection was achieved by the reversion of resistance to chemotherapy re-treatment, which the DOX-containing treatment regimens potently induced in the absence of DCT.

**Summary/Conclusions:** The development of curative treatment combinations requires deep understanding of how non-genetic factors synergize with cancer genetics to drive intra-tumor heterogeneity, which is key for tumor escape/ disease recurrence. Our detailed analyses of the heterogeneous dynamics among single-cell lineages in AML, following different treatment regimens with apparently similar global impact, represent an important step in dissecting kinesis-dependent aspects that go beyond the genetic level. Critically, these studies directly provide the rationale for combining standard chemotherapy with administration of hypomethylating drugs to target AML. The mechanism is prevention of the development of chemotherapy resistance (mediated by selective relapse of a specific set of predetermined sub-lineages) - by partially randomizing which sub-lineages that emerge to drive relapse when DCT is added to the chemotherapy. Maintaining the chemosensitivity of relapsing AML would represent a paradigm shift, turning the currently often lethal recurrences into survivable/ repeatedly clinically manageable episodes of a type of chronic leukemic disease.

**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) can improve prognostic of a possible relapse during the remission. Traditional methods for measuring minimal residual disease (MRD) in AML, such as oncoparametric 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+ patients have a long survival, which indicates that the sensitivity and specificity of traditional methods for MRD are insufficient to detect low 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 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 Tor rent 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=10, NPM1n=19, 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 specificity and sensibility calibration curves.

**Results:** We analyse 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^-6) by ROC curve with a sensibility of 0.5 for DFS and 0.571 for OS, and a specificity of 0.92 for DFS and 0.897 for OS; thereby the 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:8.37-37.15) (see figure 1). These results support the usefulness of MRD evaluation in patients with AML by NGS in the context of molecular biology studies.
E894

**GENE RATION OF NEW CELLULAR MODELS FOR THE STUDY OF PEDIATRIC NON DOWN SYNDROME ACUTE MEGAKARYOBLASTIC LEUKAEMA BASED ON HUMAN PLURIPOTENT STEM CELLS**

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**Background:** Acute megakaryoblastic leukaemia (AMKL) is a rare and complex type of Acute Myeloid Leukaemia (AML), more frequent in children than in adults, characterized by the accumulation of immature megakaryoblasts and thrombocytopenia. Paediatric AMKLs are classified in Down Syndrome AMKL (DS-AMKL) with a good prognosis; and AMKL non-related to Down Syndrome (non-DS-AMKL), a more aggressive disease with a mortality rate close to 80%. There is a limited amount of research done on infant non-DS-AMKL. Due to its low incidence and early human hematopoiesis, we were interested in studying the role of normal hematopoietic cells as model systems. In this project, we aim to use human hPSCs expressing non-DS-AMKL-associated fusion oncogenes as cellular models for this leukaemia, to study the molecular and cellular pathways involved in the development of pediatric non-DS-AMKL.

**Methods:** Generation of human models of non-DS AMKL using hPSCs: 1. Generation of hPSCs with the oncogenic fusion proteins RBBM15-MKL1, CBFAB2-T3-GLIS2 and NUP98-JARID1 using transduction with lentiviral vectors. 2. Generation of hPSCs with the chromosomal translocations t(1;12) and t(11;12), that generate the fusion proteins RBBM15-MKL1 and NUP98-JARID1 respectively, and the inversion of chromosome 16, that originates the fusion protein CBFAB2-T3-GLIS2. 3. Using these hPSC lines, we aim to study the impact of the fusion proteins on the hematopoietic system, the development of the hematopoietic lineage and the potential of these cells to differentiate into the three germ layers forming embryoid bodies. Using an in vitro differentiation system, we aim to study the differentiation of these cells into hematopoietic progenitors and megakaryocyte precursors. We will use colony-forming assays (CFU) to determine the function and specificity of the hematopoietic progenitors.

**Results:** Summary/Conclusions: These models will serve as platforms to discover and understand the cellular and molecular alterations caused by these oncogenes, and their impact in the development of hematopoietic cells during development. With these models, 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.

**E895**

**ASXL1 MUTATIONS IN AML ARE ASSOCIATED WITH SPECIFIC CLINICAL AND CYTOTGENIC CHARACTERISTICS**

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**Background:** Acute megakaryoblastic leukaemia (AMKL) is a rare and complex type of Acute Myeloid Leukaemia (AML), more frequent in children than in adults, characterized by the accumulation of immature megakaryoblasts and thrombocytopenia. Paediatric AMKLs are classified in Down Syndrome AMKL (DS-AMKL) with a good prognosis; and AMKL non-related to Down Syndrome (non-DS-AMKL), a more aggressive disease with a mortality rate close to 80%. There is a limited amount of research done on infant non-DS-AMKL. Due to its low incidence and early human hematopoiesis, we were interested in studying the role of normal hematopoietic cells as model systems. In this project, we aim to use human hPSCs expressing non-DS-AMKL-associated fusion oncogenes as cellular models for this leukaemia, to study the molecular and cellular pathways involved in the development of pediatric non-DS-AMKL.

**Methods:** Generation of human models of non-DS AMKL using hPSCs: 1. Generation of hPSCs with the oncogenic fusion proteins RBBM15-MKL1, CBFAB2-T3-GLIS2 and NUP98-JARID1 using transduction with lentiviral vectors. 2. Generation of hPSCs with the chromosomal translocations t(1;12) and t(11;12), that generate the fusion proteins RBBM15-MKL1 and NUP98-JARID1 respectively, and the inversion of chromosome 16, that originates the fusion protein CBFAB2-T3-GLIS2. 3. Using these hPSC lines, we aim to study the impact of the fusion proteins on the hematopoietic system, the development of the hematopoietic lineage and the potential of these cells to differentiate into the three germ layers forming embryoid bodies. Using an in vitro differentiation system, we aim to study the differentiation of these cells into hematopoietic progenitors and megakaryocyte precursors. We will use colony-forming assays (CFU) to determine the function and specificity of the hematopoietic progenitors.

**Results:** Summary/Conclusions: These models will serve as platforms to discover and understand the cellular and molecular alterations caused by these oncogenes, and their impact in the development of hematopoietic cells during development. With these models, 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.

**CHARACTERIZATION OF HEMATOLOGIC MALIGNANCIES WITH ANCHORED MURPLEX 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 a challenge, 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 levels and copy number alterations (CNA).

**Methods:** We developed AMP-based Anchor21* VariantPlex™ and FusionPlex® assays to enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification permits identification of novel gene fusions with FusionPlex and complex mutation types such as ITDs with VariantPlex assays. MBC adapters ligated to DNA and RNA fragments prior to amplification enable relative gene expression and CNA analysis.

**Results:** We show instances of gene fusion detection from open-ended amplification (AMP), including RNU7-UGA, RUNL1 and RUNL2, and show how this technology can be applied to acute unclassifiable leukemias. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL fusion, which was detected with reads originating from BCR1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected ITDs of varying sizes and insertion points, with simultaneous point mutation detection, in AML-positive blood samples. Furthermore, we show multiple mutations in various AML-positive sample types, including mutations in CEPBP. Finally, MBCs used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B Cell Lymphoma subtypes in a small cohort of samples.

**Summary/Conclusions:** Our data demonstrate that AMP-based NGS enables comprehensive detection of multiple mutation types as well as gene 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.
mutations. Moreover, ASXL1 mutations were detected in 3 of 12 patients with aberrations involving chromosome 8 (25%), 2/9 (22%) with trisomy 13, 2/11 (18%) with t(9;22) and only 1 of 22 patients with t(15;17). Multivariate logistic regression 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 del(7q) or -del7q predicted for lower frequency (OR 0.32, 95% CI 0.13-0.75).

Summary/Conclusions: Our results support the association of ASXL1 mutations in AML with advancing age and sec-AML. Association with trisomy 8 did not retain significance in multivariate analysis. Chromosome 11 aberrations emerged as a robust independent predictor. Despite the strong link with secondary AML (majority of cases post MDS), our data show inverse relationship with -7/del(7q) or -del7q. 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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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);t(8;13), t(16;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 to carry cytogenetic abnormalities were taken for routine diagnostic (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 tests, can establish whether the TLA panel is applicable as a routine procedure.

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 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);t(8;13), t(16;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 to carry cytogenetic abnormalities were taken for routine diagnostic (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 tests, can establish whether the TLA panel is applicable as a routine procedure.

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 with high sensitivity of the detection 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 (nocto-neurotoxic) genes, secondary disease or therapy resistance at diagnosis and we analyzed the Overall Survival (OS) of our patients with alterations in these genes is significantly lower than control group, with a median OS of 3 months vs 27 months in the control group.

Summary/Conclusions: Our study shows that losses in necroptosis pathways are an uncommon alteration in AML, prevalent in old population. Moreover, we hypothesize that the loss of genes involved in necroptosis could be a real mechanism of tumor immune-escape and could be a rational to select patients that high probability to be resistant at chemotherapy promoting ICD mechanism.

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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: see figure 1.

Figure 1. We performed a custom-targeted sequencing panel of 32 genes (all coding regions) implicated in leukemia prognosis, including ASXL1, CBL, DNMT3A, EPO, ETV6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, LNK, MPL, NRAS, PHF6, PRPF40B, PTEN, RUNX1, SF1, SF3A1, SF3B1, SRSF2, TET2, TP53, U2AF35, VHL, ZRSR2, and CALR, by Ion Torrent Proton System-Thermo Fisher. Primary tumor-refractory (n=8) and primary tumor-relapsed (n=17) samples pairs from 25 AML patients treated according PETHEMA AML clinical protocols were sequenced; in addition FLT3-ITD was detected by GENSCAN and NPM1 mutation was detected by PCR. We analyse the evolution of level of VAF, to measure the prevalence of somatic mutations between diagnosis and resistance status (relapse or refractory).

Results: see table.

Methods: see table.

Figure 1. 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 re-collected 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 Calcusyn software.

Discussion: see table.

E899

E900
IDENTIFICATION OF NOVEL THERAPEUTIC DRUGS IN DISTINCT PEDIATRIC AML SUBTYPES BY TARGETING EPIGENETIC REGULATORS

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Background: Treatment protocols for pediatric acute myeloid leukemia (AML) are chemotherapy-based, including high-dose cytarabine. While >90% of patients reach clinical remission, there is still a high relapse rate of ~30%, with overall survival rates of 60-70%. Therefore, better risk-classification at diagnosis and alternative treatment strategies are warranted. There is increasing evidence that epigenetic deregulation is involved in the initiation and progression of cancers, including AML. Epigenetic processes are required for hematopoiesis and epigenetic regulators are frequently translocated (MLL) or mutated (EZH2) in AML. Following this, deregulated epigenetic pathways could be used for targeted therapy and provide an alternative approach to improve pediatric AML therapy.

Aims: To identify new therapeutic drugs in pediatric AML by using an 80-compound screen containing inhibitors of epigenetic regulators, including histone writers (which deposit post-translational modifications (PTMs) on histones), readers (binding of PTMs) and erasers (removal of PTMs).

Methods: Cell lines used in this study are THP-1 (t(9;11)), Kasumi-1 (t(8;21)) and CMK (Down’s syndrome with GATA1 mutation), reflecting distinct pediatric AML entities and a differential response to treatment with cytarabine. Cells were treated for 72hrs followed by analysis of cell viability and apoptosis based on Hoechst, Draq7 and Calcein Green staining. The expression of three candidate compounds were further investigated in triplicates at several concentrations for their effect on cell viability (Annexin V/PI staining), cell cycle, morphology, and apoptosis. As controls, normal myeloid precursor cells derived from Kasumi-1, CMK and THP-1, resp. While inhibition by LMK235 resulted in an increase of cells in S-phase and G2/M. Among the differential effects of the combination of alvocidib, cytarabine, and daunorubicin in Kasumi-1, CMK and THP-1, resp. Inhibition by LMK235 at an IC50 of 0.1µM, 0.13µM and 0.425µM in Kasumi-1, CMK and THP-1, resp. While inhibition by LMK235 resulted in an immediate increase of apoptosis, Bromosporine-treated cells retained in G1 through cell cycle arrest and, interestingly, in vitro, treatment of CMK cells with LMK235 results in a greater 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, indicated by a 10-fold increase in concentration required for NSC3852-induced apoptosis. Interestingly, upon addition of LMK235 to 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 cycle arrest and reduced cell proliferation. The differential 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.

ALVOCIDIB SYNERGIZES WITH CYTARABINE AND DAUNORUBICIN (7+3) IN PRECLINICAL MODELS OF ACUTE MYELOID LEUKEMIA

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Background: Although survival of patients with acute myeloid leukemia (AML) has increased in the years 1975-2011, the 5-year, overall survival of patients with AML remains unacceptably low at an estimated 26% (2011). 7+3 treatment (cytarabine 100-200mg/m² + anthracycline [daunorubicin 60-90mg/m²/day or idarubicin 12mg/m²/day]) remains the standard induction therapy for AML patients, and has persisted largely unaltered for more than 30 years. There is a significant unmet clinical need for improved therapeutic options in patients with AML. Alvocidib is a CDK9 inhibitor currently in development for the treatment of patients with AML. Alvocidib has previously been studied as part of the ACM regimen, a combination regimen incorporating the time-to-remission-guided administration of alvocidib, cytarabine, and mitoxantrone, specific in multiple Phase 1 and 2 clinical trials. The ACM regimen has achieved significant improvements in complete response (CR) rates versus 7+3 in previously untreated intermediate and high-risk AML patients. The rationale for time-sequential administration of the ACM regimen was developed with an incomplete understanding of alvocidib’s mechanism of action. TST was based on the expectation that alvocidib would synchronize cells, capitalizing on the S-phase specific activity of cytarabine in the combination. However, rather than cell-cycle inhibition, alvocidib potently induces apoptosis by inhibiting the expression of key anti-apoptotic proteins via CDK9/RNA polymerase II, including MCL1. With this understanding, we reasoned that alvocidib would also 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 assays was used for cell viability and apoptosis, respectively. We used mouse xenograft models in the format of an MV4-11 xenograft mouse model. Following manufacturer’s protocol. We used RT-PCR to measure mRNA expression of MCL1 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 an MV4-11 xenograft mouse model.

Results: Single agent IC50 values of cytarabide, 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 no good induction with single agent daunorubicin or cytarabine. In the combination, we observed a very 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 MCL1 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 studies, along with the pan-cancer cellular and mouse xenograft studies, further support the potential of alvocidib as a time-sequential addition to the 7+3 regimen as a means to enhance the activity of the standard of care. In this study, alvocidib demonstrated both preclinical and clinical activity with increased efficacy when added to the 7+3 regimen as a means to enhance the activity of the standard of care.
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-cell mouse model, and from 21 days to 50 days in the BNML rat model. Additionally, the IFNα-Le (0.8x10^6 IU/kg) and VPA (400mg/kg) combination treatment indicated a tendency to increased survival in the BNML model. However, IFNα-Le monotherapy (1x10^6 IU/kg) decreased survival in the MOLM-13Luc+ model.

**Figure 1.**

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.

E904

KEVETRIN: PRECLINICAL STUDY OF A NEW COMPOUND IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemia (AML) is a heterogenous 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 Cellceutix, with the ability to target both wild type and mutant p53 tumors.

Aims: The aim of this project is to explore cellular and molecular alterations induced by Kevetrin, focusing on its role in the p53 pathway.

Methods: Kevetrin was kindly provided by Cellceutix, dissolved and stored at 4°C in sterile water in a 600 μg/ml stock solution, and diluted in medium immediately before use [concentration range in use 15-60μg/ml]. Cell lines, MOLM-13 and KASUMI-1, were cultured in RPMI 1640 supplemented with 20% heat inactivated fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. After 24 and 48 h of treatment MTS, Annexin-V, TUNEL, 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 deregulated 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.

E905

CLEARANCE OF ‘DRIVER-COSMIC’ MUTATIONS POST CR1 WITH OPERATING RUNX1 L565 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 ≥1% at diagnosis and VAF of ≥1.5% 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).

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-CS) 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-CS mutation in the remission cohort (8x) revealed that 4 patients had persistent DNTM3A-25457242, 1 had DNTM3A-25457243, 2 had RUNX1-36259324/L565 and 1 had CBL-119149011. As DNTM3A mutations are considered to occur in pre-leukemic disease.
stem cells contributing to clonal haematopoiesis (Askush et al, Nature 2014; Genovese et al, NEJM 2014); this led us to study the distribution of RUNX1 gene variants in an additional 119 AML diagnostic samples. 34 patients (21%) harboured RUNX1 mutation, of which 5 had RUNX1_L56S that were often associated with D-C mutations (4 of 5 cases). Finally, we evaluated kinetics of D-C in 40 MDS cases of which 34 had chronic MDS and 6 had secondary AML (sAML). No significant difference was observed in the number of patients with persistent D-C mutation in the 2 subgroups (chronic MDS: 16 of 19 (84.2%); sAML: 5 of 5 (100%); P-value: 1.000).

Table 1.

Summary/Conclusions: Clearing of ‘Driver-COSMIC only’ mutations while RUNX1_L56S persists is unlikely to contribute towards disease progression in AML.

Acute myeloid leukemia - Clinical

E906

PROGNOSTIC SIGNIFICANCE OF FLT3 STATUS, CYTOGENETIC, ECOG AND 50% BLAST DECREASE IN PRIMARY REFRACTORY OR EARLY RELAPSED AML PATIENTS BEFORE SALVAGE THERAPY

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Background: Prognosis of relapsed/refractory acute myeloid leukemia (R/R AML) is unfavorable with a long term overall survival around 10%. Thus, management of R/R AML represents one of the most difficult challenges. Because allogeneic-Hematopoietic Stem Cell Transplantation (allo-HSCT) is considered as the best treatment for this category of patients, to determine which patient will benefit from this cumbersome strategy is a crucial issue. A better understanding of the mutational status, cytogenetic, histological and clinical findings of early R/R AML patients and their outcomes could help treatment decisions, particularly for those who allo-HSCT is considered as the best therapeutic option.

Aims: The objective of this study is to determine prognostic factors and develop a prognostic score using usual mutational status, cytogenetic, histological and simple clinical variables in R/R AML patients before salvage treatments.

Methods: In this retrospective study in two hematological departments (Hospices Civils de Lyon and CHU of Toulouse), we evaluated clinical, biological, histological, cytogenetic and current mutational status of early R/R non APL AML patient between age from 18 to 70 years. Univariate and multivariate analysis were performed and we developed a prognostic score based on the independent prognostic parameters from Cox model.

Results: From January 2009 to May 2016, 58 patients presenting early relapse and primary refractory AML were analyzed. Overall Survival (OS) and Progression Free Survival (PFS) median were 9 and 2 months respectively. In univariate analysis, cytogenetic findings (unfavorable groups), unfavorable ECOG (>2), FLT3 positive status and <50% blast decrease (between induction and R/R assessment) independently predicted poor OS and were identified as significant prognostic parameters of OS (p=.037, p=.0084, p=.0452, p=.0071 respectively). In multivariate analysis, these last four criteria confirmed their worst prognostic impacts (p=.015, p=.017, p=.026, p=.015 respectively) and were used to create a five groups prognostic score. Better OS were statistically observed for patient with score 0 or 1 compared to 2, 3 or 4 (2-years OS 48% and Not Reached respectively, p=.0104) (Figure 3).

Summary/Conclusions: Our prognostic score based on simple and usual data: FLT3 status, cytogenetic, ECOG and percentage blast decrease found distinct groups with statistically different outcomes. Basically, the higher is the score, the worst is the OS. This new score is a valuable, simple and useful score for the therapeutic salvage management of AML patients presenting early relapse and primary refractory.
PRELIMINARY RESULTS FROM A PHASE 1 STUDY EXAMINING THE NOVEL BCL-2 INHIBITOR S55746/BCL201 AS SINGLE AGENT IN PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH RISK MYELODYSPLASTIC SYNDROME

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Background: Novel and effective therapeutic options for patients (pts) with advanced acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are limited. Targeting the 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 unfit for intensive chemotherapy (IC)), or MDS failing prior therapies.

Methods: A phase I study (EUDRACT 2014-002559-24, NCT02902541) 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 number of prior therapies 2 (range 0-6), ECOG ≤2, and median WBC 3.1 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%. 28 pts were R/R AML, 2 pts were elderly AML unfit for IC, or 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 3.1 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%.

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 (18%)), 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). 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-compartamental 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

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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 (Papaemmanuil, NEJM 2016) suggest that the coexistence of additional gene mutations (e.g. DNTM3A, 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 quantification 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 multiparametric flow cytometry (MFC) might represent an alternative tool to discriminate different prognoses 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 concomitantFLT3-ITD mutation; 51/66 (77%) NPM1 mutated cases had a normal diploid karyotype. Upon full hematological recovery after consolidation cycle, counting, by MFC, ≥3·5×1010·0.0335 residual leukemic cell (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, MRD negative/NPM1 WTs had a lower Cumulative Incidence of Relapse (CIR) as compared to MRD positive/NPM1 mut patients who had a higher Cumulative Incidence of Relapse (CIR) as compared to MRD negative/NPM1 WTs (25% vs 80%). We also evaluated the impact of autologous (AuSCT) or allogeneic (ASCT) transplantation on the outcome of MRD positive/NPM1 mut patients. The overall survival (OS) was significantly higher for patients submitted to ASCT (no=14) as compared to those (no=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 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 mut 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, AND PD-L2) ON BONE MARROW T CELLS IN ACUTE MYELOID LEUKEMIA


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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.
(SCT). However, evidence regarding T cell phenotypes for patients with AML is sparse.

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), persistence after SCT (P=0.0025 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-L1 and PD-L2 expression on leukemic cells at time of diagnosis was higher in secondary AML transformed from myelodysplastic syndrome than in de novo AML (P=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.

E910

ACUTE LEUKEMIA IN HIV PATIENTS: EPIDEMIOLOGY, THERAPEUTIC STRATEGY AND PROGNOSIS

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Background: Data on HIV patients with acute leukemia (AL, acute myeloid leukemia (AML) or non Burkitt acute lymphoid leukemia (ALL)) are very poor especially on their outcome. Treatment of acute leukemia usually depends of patient-related prognostics factors and disease related prognostic factors. Because HIV patients are considered frail, they are always excluded of therapeutic protocols. There are no guidelines for their treatments.

Aims: Our aim was to precise the epidemiology, the best therapeutic strategies as well as patient’s prognostics, and to compare their outcome to those of seronegative patients with AL.

Methods: We conduct a retrospective national multicentric study. HIV positive patients with a diagnosis of AML or non Burkitt-ALL between January 2000 and February 2016 were included. We compared HIV patients’ outcome to those of seronegative patients with AL after a propensity score matching.

Results: 47 HIV patients with a diagnosis of AL (42 AML and 5 ALL) were included. AL incidence in HIV patients (HIVP) is not different than in general population but AL occurred earlier (49.29 years [44.21 ; 57.47]) and AL are more frequent (42.55%). With a global and multidisciplinary approach these patients can be treated with intensive chemotherapy resulting on good efficiency (complete remission [CR]=84.38%) and tolerance. Based on a multivariable model, only absence of CR was associated with hazard of death (p=0.01). 8 patients (17,02% ; 7 AML and 1 ALL) received a hematopoietic stem cell transplantation. HIVP with AL 2 years overall survival (OS) was 29% C195% [15 ; 54] for AML and 40% C195% [14 ; 100] for ALL. There was no difference in OS between our HIVP and seronegative controls with AL after propensity score matching (HR=1.347 [0.6846-2.796]; p=0.42).

Table 1.

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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 FOR INTENSIVE CHEMOTHERAPY

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WITH ACUTE MYELOID LEUKEMIA FIT FOR INTENSIVE CHEMOTHERAPY
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 AML. Ten-day regimen of decitabine induces a higher response in newly diagnosed older patients with AML considered unfit for intensive chemotherapy. But this 10-day regimen has not been tested in older fit AML before.

Aims: To investigate the efficacy and safety of the 10-day decitabine regimen in older fit AML prospectively.

Methods: Twenty-one older patients (>60 years old) with newly diagnosed intermediate or adverse cytogenetic risk group AML, considered fit for intensive chemotherapy, were enrolled in a prospective clinical trial. These patients refused to take intensive chemotherapy. All patients were treated with at least one course of 10-day decitabine consolidation. The primary endpoint was achieved less than 5% bone marrow blasts were subsequently treated with 5-day decitabine courses as maintenance therapy. Median age was 64 (range 60-74) years. There are 5 patients with (23.8%), 10 (47.6%), 6 (28.6%) in favorable, intermediate and poor-risk group, respectively, based on the NCCN guideline. All patients had an Eastern Cooperative Oncology Group performance status of 0-1.

Results: The overall response rate (ORR) was 57.1%, including 52.4% CR. There are no significant differences between responders and non-responders, in the following parameters, including age, LDH, DNMT3A mutation, white blood cells count in peripheral blood, or bone marrow blasts percentage. Nineteen patients achieved negative residual disease (MRD) status, as measured by multi-parametric flow cytometry, in pts with AML who receive indoximod in combination with standard of care (SOC) chemotherapy after completion of the 1st cycle of consolidation, and before maintenance or proceeding to allogeneic stem cell transplantation (allo-HSCT).

Summary/Conclusions: Indoximod is given orally every 8 hours starting on day 8 of induction onward. Indoximod is an inhibitor of the IDO pathway, into conventional remission, and before maintenance or proceeding to allogeneic stem cell transplantation (allo-HSCT). This is a phase 1b / randomized phase 2a trial of indoximod in combination with decitabine for upfront treatment of patients with newly diagnosed acute myeloid leukemia (AML): Phase 1 report.

E912 INDOXIMOD IN COMBINATION WITH IDRACUBYN AND CYTARABINE FOR UPFRONT TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML): PHASE 1 REPORT

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Background: AML cells can acquire immune evasion and tolerance through overexpression of IDO, which targets immunomodulatory effects through tryptophan (Trp) catabolism and kynurenine production. By degrading Trp, IDO shifts the balance from a Trp-rich environment leading to immune system suppression. We hypothesized that incorporation of indoximod, an inhibitor of the IDO pathway, into conventional remission induction and consolidation would be well tolerated without adding significant toxicity and may improve clinical outcomes of patients (pts) with newly diagnosed AML.

Aims: The primary objective of the phase 1 portion of the trial is to characterize the Asahina and Allergy, Chevy Chase, Maryland, United States, 6Georgia Cancer Center and Department of Pediatrics, Medical College of Georgia, Augusta, Hematologics Inc., Seattle, 7NewLink Genetics Co., Ames, United States

Methods: Twenty-one older patients (>60 years old) with newly diagnosed intermediate or adverse cytogenetic risk group AML, considered fit for intensive chemotherapy, were enrolled in a prospective clinical trial. These patients refused to take intensive chemotherapy. All patients were treated with at least one course of 10-day decitabine consolidation. The primary endpoint was achieved less than 5% bone marrow blasts were subsequently treated with 5-day decitabine courses as maintenance therapy. Median age was 64 (range 60-74) years. There are 5 patients with (23.8%), 10 (47.6%), 6 (28.6%) in favorable, intermediate and poor-risk group, respectively, based on the NCCN guideline. All patients had an Eastern Cooperative Oncology Group performance status of 0-1.

Results: The overall response rate (ORR) was 57.1%, including 52.4% CR. There are no significant differences between responders and non-responders, in the following parameters, including age, LDH, DNMT3A mutation, white blood cells count in peripheral blood, or bone marrow blasts percentage. Nineteen patients achieved negative residual disease (MRD) status, as measured by multi-parametric flow cytometry, in pts with AML who receive indoximod in combination with standard of care (SOC) chemotherapy after completion of the 1st cycle of consolidation, and before maintenance or proceeding to allogeneic stem cell transplantation (allo-HSCT).

Summary/Conclusions: Indoximod is given orally every 8 hours starting on day 8 of induction onward. Indoximod is an inhibitor of the IDO pathway, into conventional remission, and before maintenance or proceeding to allogeneic stem cell transplantation (allo-HSCT). This is a phase 1b / randomized phase 2a trial of indoximod in combination with decitabine for upfront treatment of patients with newly diagnosed acute myeloid leukemia (AML): Phase 1 report.

E913 PHASE III STUDY OF MEK INHIBITOR (MEK-162; BINIMETINIB) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MYELOID MALIGNANCIES

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Background: Activation of the mitogen-activated protein kinase (MAPK) signaling (RAS/RAF/MEK/ERK pathway) promotes growth and inhibits apoptosis of hematopoietic cells. Inhibition of MEK/MAPK pathway has shown antiproliferative effects in acute myeloid leukemia (AML) blasts. MEK-162 is an oral, potent, selective allosteric, ATP-non-competitive inhibitor of MEK1 and 2.

Aims: To study the efficacy and safety of MEK-162 in patients with advanced myeloid malignancies.

Methods: Patients with relapsed/refractory AML, not candidates for intensive chemotherapy, and patients with high risk myelodysplastic syndrome (MDS) who were resistant/intolerant to standard treatment including stem cell transplant were treated with MEK-162 twice daily every 28 days. Patients in the expansion phase had to be RAS mutated. The primary endpoint was overall response rate (ORR=CR+CRI) after 1 cycle of therapy. Survival was estimated using the Kaplan-Meier method. Safety analysis included all patients who had received at least 1 dose of MEK-162. MEK-162 dose escalation followed a 3+3 design; phase 2 had built in futility/toxicity boundaries. 45mg twice daily is the final dose level for expansion phase.

Results: Sixteen patients were treated (escalation=7; expansion=9): 14 AML and 2 MDS. Median age was 62 years (31-85). 56% were male; 94% had a performance status of 1-2. Median number of prior therapies was 4 (1-6). 3/16 (19%) patients had complex karyotype. 11/69 (16%) patients were RAS mutated. All 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 (13%) and electrolyte abnormalities (14%). No dose limiting toxicity was reported.

Summary/Conclusions: MEK-162 shows a tolerable safety profile with an ORR of 10%. The study is currently on-going. Additional studies involving combination of MEK-162 with RAF and PI3 kinase inhibitors are ongoing.

E914 HAPLOIDENTICAL TRANSPLANTATION IS SAFE AND EFFECTIVE FOR OLDER PATIENTS WITH AML/MDS

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Background: Acute myeloid leukemia (AML) is more common in the older population. Haploidentical stem cell transplantation (haploSCT) is a potentially cur-
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 thiopeta 5mg/kg or 2GyTBI. Characteristics of these patients are presented in Table 1.

Results: Median age was 61 years (range 55-69), 22 patients (51%) were in CR1/2, 16 patients (37%) had poor-risk cytogenetics, and median HCT-CI was 2 (range 0-11). Reduced melphalan regimen (100mg/m²) was used in 29 pts (67%). Donors were children in 35 (81%) or siblings 10 (19%) patients.

Median follow-up was 19 months (range 6-49). One patient died prior to engraftment. Forty-two patients engrafted the donor cells (100%). Median time to neutrophil and platelet engraftment was 19 (13-28) and 28 (15-117) days. Day 30 chimerism was 100% donor in 38 patients (88%). The cumulative incidence (CI) of grade 2-4 and 3-4 aGvHD at 6 months post-transplant was 35% and 5% while CI of cGvHD at 2 years post-transplant was only 9%. The 2-year overall survival (OS) and progression-free survival (PFS) was 42%, and relapse rate was 24%. Cumulative non-relapse mortality (NRM) was 21%, 30% and 34% at day 100, 1 year, and 2 years post-transplant. Patients in CR1/2 had 2-year NRM and relapse rate of 23% and 14%, and OS was 61%. The 2-year OS for patients in CR1/2 with intermediate/favorable-risk cytogenetics was 73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good/intermediate cytogenetics (HR:0.2, p=0.01), and donor age greater than 40 (Figure 1).

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>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>25 (58%)</td>
</tr>
<tr>
<td>MDS/AML MDS</td>
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<td>MDS</td>
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<tr>
<td>Follow-up</td>
<td>19 (6-49)</td>
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<tr>
<td>Disease status</td>
<td></td>
</tr>
<tr>
<td>CR1/2</td>
<td>22 (51%)</td>
</tr>
<tr>
<td>CR</td>
<td>31 (69%)</td>
</tr>
<tr>
<td>Disease stage</td>
<td></td>
</tr>
<tr>
<td>CR1/2</td>
<td>22 (51%)</td>
</tr>
<tr>
<td>CR</td>
<td>31 (69%)</td>
</tr>
<tr>
<td>Donors</td>
<td></td>
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<tr>
<td>Child</td>
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<tr>
<td>Sibling</td>
<td>8 (19%)</td>
</tr>
<tr>
<td>Donor site</td>
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<tr>
<td>Female/donor</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>Male/receptor</td>
<td>10 (23%)</td>
</tr>
<tr>
<td>Sex mismatch</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>Male/male</td>
<td>10 (23%)</td>
</tr>
</tbody>
</table>

Figure 1. Results: Median age was 61 years (range 55-69), 22 patients (51%) were in CR1/2, 16 patients (37%) had poor-risk cytogenetics, and median HCT-CI was 2 (range 0-11). Reduced melphalan regimen (100mg/m²) was used in 29 pts (67%). Donors were children in 35 (81%) or siblings 10 (19%) patients.

Summary/Conclusions: Our data show that MRD assessment at different time-point reduces the risk of late relapse by demonstrating the persistence of disease for different populations. The percentage of MFC-MRD negativity is higher than 90%, and patients with MFC-MRD negativity at TP1, TP2, TP3 (NPM-MRD) were more likely to achieve treatment control. The probability of achieving MFC-MRD negativity at TP2 was only influenced by ELN risk group (p<0.05). In the whole cohort, 2 years OS was 60.2% (median not reached). Multivariate Cox-Proportional Hazard model showed that MFC-MRD >0.1% at TP2 was the strongest predictor of higher risk of death, whereas MFC-MRD <0.025 at TP1 was the strongest predictor of long survival (Figure 1). 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-color MFC analysis (and 8-color from 2013). To define the MFC-MRD positivity two cut-offs were considered: 0.2×10−4 residual leukemic cells (>0.025%) or a threshold of 1×10−3 residual leukemic cells (>0.1%). For patients carrying NPM1-gene mutation NPM1 expression levels at TP1, TP2, TP3 (NPM-MRD) were analyzed. A reduction >3.5 log of NPM1 transcript at TP1 was considered optimal as per our published experience. For patients presenting WT1 over-expression at diagnosis WT1-MRD was evaluated at TP1, considering WT1 negativity with a cut-off of WT1 c/b/104 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 >90% and >0.1% increased from TP1 to TP2. The probability of achieving MFC-MRD negativity at TP2 was only influenced by ELN risk group (p<0.05). In the whole cohort, 2 years OS was 60.2% (median not reached). Multivariate Cox-Proportional Hazard model showed that MFC-MRD >0.1% at TP2 was the strongest predictor of higher risk of death, whereas MFC-MRD <0.025 at TP1 was the strongest predictor of long survival (Figure 1). Multivariate Cox-Proportional Hazard model showed that MFC-MRD >0.1% at TP2 was the strongest predictor of higher risk of death, whereas MFC-MRD <0.025 at TP1 was the strongest predictor of long survival (Figure 1). Thirty-five patients carried NPM1-mutation. Two-years OS for NPM1-mut patients showing more or less than 3.5 log 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-points reduces the risk of late relapse by demonstrating the persistence of disease for different populations. The percentage of MFC-MRD negativity is higher than 90%, and patients with MFC-MRD negativity at TP1, TP2, TP3 (NPM-MRD) were more likely to achieve treatment control. The probability of achieving MFC-MRD negativity at TP2 was only influenced by ELN risk group (p<0.05). In the whole cohort, 2 years OS was 60.2% (median not reached). Multivariate Cox-Proportional Hazard model showed that MFC-MRD >0.1% at TP2 was the strongest predictor of higher risk of death, whereas MFC-MRD <0.025 at TP1 was the strongest predictor of long survival (Figure 1). Thirty-five patients carried NPM1-mutation. Two-years OS for NPM1-mut patients showing more or less than 3.5 log 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.
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 registered prospective randomized clinical trial AML 2010-01(201002204). We reviewed the data from bone marrow flowcytometry before the first and second course of consolidation therapy, in which the CD34+/CD38+/CD117+/HLA-DR−/CD13+CD33+ progenitor cell percentage in the bone marrow was analyzed. Plasma recovery time and time of neutrophiia 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 neutrophil recovery after chemotherapy. Multivariate analysis demonstrated that P cells were an independent negative factor affecting neutrophil recovery capability for both first and second courses (p=0.015; p=0.036, respectively).

Summary/Conclusions: Our results indicate that CD34+/CD38+/CD117+/HLA-DR−/CD13+CD33+ cells before each course of chemotherapy is associated with 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.

E917
MICRONORAS (miRS) IN HIGH RISK PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) AMONG PATIENTS WHO UNDERGO STEM CELL TRANSPLANTATION FOR RELAPSE
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1Oncohaematology, Bambino Gesù Children Hospital, Roma, Italy, 2Medical Genetics, 3Genomic Shared Resources, Ohio State University, Columbus (OH), United States, 4Oncohaematology-Pediatrics, University of Padova, Padova, Italy

Background: Despite recent progresses made in the treatment of acute myeloid leukemia (AML) of childhood, the cure rates of high-risk subtypes remain low. Indeed, patients harboring FLT3-ITD mutations or 11q23 translocations (MLL rearrangements) are still characterized by a poor prognosis, mainly due to leukemia 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 further studied.

Aims: The aim of this study is to evaluate baseline levels of selected cytokines, cytokine receptors and adhesion molecules and their relationship with prognosis in newly diagnosed AML patients.

Methods: A total of 75 AML patients, age 52.9±13.0 years, median 58.5 years, 44 female were, were studied in the period 2010-2015. Only patients with minimal follow-up of 1 year were included. All patients were induced with “3+7” induction chemotherapy consisting of Cytarabine 100mg/m2 per day for 7 consecutive days and Daunorubicin 90mg/m2 for the first 3 days of therapy in younger patients. Since the beginning of 2015, the induction dose of Daunorubicin used has been 60mg/m2 even in younger patients, according to recent evidence-based data modifications. Those who failed to achieve CR were given FLG+ ld savage following by allogeneic stem cell transplantation in younger and fit patients. In CR, the patients were treated either with HI-DAC consolidations and/or allogeneic stem cell transplantation in younger and fit patients. A total of 39 patients underwent allogeneic stem cell transplantation. We evaluated serum levels of the following 29 analytes: interleukins (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15), IL-1β, Pneumonin Activity, Macrophage Inflammatory Protein-1α, Monocyte Chemotactic Protein-1, Tumor Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor, E-selectin (E-SEL), P-selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Matrix Metalloproteinase-9, soluble IL-2 receptor (sIL-2R) and soluble receptors for IL-6 (sIL-6R) and TNF-α type I and II receptors, adhesion molecules: E-selectin (E-SEL) and P-selectin (P-SEL), ICAM-1, VCAM-1, sIL-2Rα and sTNF-α.

Results: IL-1β and sIL-2Rα were significantly higher in patients who failed to achieve CR by induction therapy had higher IL-7 levels, which was not significant after Bonferroni correction (P=0.0913). Inferior PFS was associated with higher sIL-2Rα (P=0.0525). Inferior OS was significantly associated with higher P-SEL (P=0.0003), higher sIL-2Rα (P=0.0029), higher age (P=0.0366) and possibly with higher TNFR-1 (P=0.0611). Age has not correlated with any of the other analyzed analyte. TNFR-1 correlates with TNF-α (P=0.0045), but not with TNF-α. The sIL-2Rα did not correlate with IL-2. Only IL-6 and ICAM-1 were significantly influenced by CRP levels.

Summary/Conclusions: Better understanding of the cancer microenvironment is a sine qua non for development of new treatment approaches. Our results suggest that cytokine levels may be useful markers to predict the treatment outcome and thus should be further investigated as possible therapeutic targets. The work was supported by a long-termorganisation development plan 101 (FMHS) and by MH CZ – DRO (UHKK, 0179908).
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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1Clinic of Hematology, Department of Internal Medicine (DIMI), University of Genoa, IRCCS AOu San Martino-IST, Genova, Italy

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 (< 2008; 2008-2010; 2011-2013; 2014-2016) and to recognize factors possibly leading to this result. 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 25 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 deaths. A standard treatment of low dose cytarabine (LD-AraC) or using hypomethylating therapy is not satisfying enough. Polish Adult Leukemia Group’s (PALG) studies showed, that addition of cladribine to daunorubicine and cytarabine increases the complete remission rate and improves overall survival in younger patients with AML. We also proved effectiveness of cladribine combined with high dose AraC and mitoxantrone in relapsed and refractory AML (1, 2). 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. days 1-10 every 28 days followed by two 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 (PR). Median number of cycles to obtain CR was 2 (range 1-3), 16% of patients did not respond to treatment. The regimen was well tolerated without 4-week and 8-week mortality. The main reason of death 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.
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-gene panel using a series of contrived 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 5,265x to 7,680x. Comparison of FLT3 analysis of the small panel to amplicon based NGS assay and CE, FLT3-ITD showed complete concordance in clinical samples - and showed a strong linear relationship between the two methodologies, indicating feasibility of the assay using the international ISO13485 design control standards makes them suitable for regulatory approval worldwide.

E922
EFFICACY BY OUTPATIENT VS INPATIENT ADMINISTRATION OF CONSOLIDATION: SUBGROUP ANALYSIS OF A PHASE 3 STUDY OF CPX-351 IN OLDER ADULTS WITH NEWLY DIAGNOSED HIGH-RISK ACUTE MYELOID LEUKEMIA

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Background: The CPX-351 liposomal formulation delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin preferentially to leukemia cells. CPX-351 has demonstrated significantly improved overall survival (OS) versus cytarabine/daunorubicin (7+3) in a randomized, open-label, phase 3 study in patients aged 60 to 75 years with newly diagnosed, high-risk AML (Lancet, et al. ASCO 2016). In contrast to the 7+3 regimen, which includes cytarabine continuous infusion for 7 days, CPX-351 is administered as a 90-minute infusion and thus has the potential to be given in the outpatient setting.

Aims: The current analysis of the phase 3 trial assessed the number of patients getting treated in the outpatient setting and their outcomes.

Methods: Enrolled patients were randomized 1:1 to receive 1 to 2 induction cycles of CPX-351 or 7+3; patients with complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles (CPX-351: 65 units/m² [cytarabine 65mg/m² + daunorubicin 28.6mg/m²] on Days 1 and 3; 7+3: cytarabine 100mg/m²/day x 5 days + daunorubicin 60mg/m² on Days 1 and 2). The site of administration was not protocol defined.

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Inpatient</th>
<th>Outpatient</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CPX-351</td>
<td>7+3</td>
</tr>
<tr>
<td>Consolidation, n (%)</td>
<td>26/49 (49)</td>
<td>30/32 (98)</td>
</tr>
<tr>
<td>Median Os, months</td>
<td>14.72</td>
<td>25.43</td>
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<td>Hazard ratio (95%)</td>
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<td>0.50/0.01, 1.13</td>
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<td></td>
<td>9/22 (92%)</td>
<td>12/10 (100%)</td>
</tr>
<tr>
<td>Median Os, months</td>
<td>12/10 (100%)</td>
<td>25.43</td>
</tr>
<tr>
<td>Hazard ratio (95%)</td>
<td>Not reached</td>
<td>12/10 (100%)</td>
</tr>
<tr>
<td></td>
<td>0.41 (0.03, 2.56)</td>
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</tr>
</tbody>
</table>

Results: Few patients received induction as outpatient therapy (CPX-351 n=3/153 and 7+3 n=1/151 in each cycle). A total of 49/153 patients in the CPX-351 arm and 32/151 patients in the 7+3 arm received consolidation. In contrast to the induction cycles, a substantial proportion of patients received CPX-351 in the outpatient setting (consolidation 1: n=25/49 [51%]; consolidation 2: n=2/12 [17%]). Consolidation was associated with numerical improvement in median OS versus 7+3, irrespective of administra-

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 pat-

Methods: The CONNECT MDS/AML Disease Registry (NCT01688801) is a comprehensive, observational cohort study of patients with newly diagnosed AML (≥25 years) or myelodysplastic syndrome (MDS). All clinical decisions are made by the treating clinicians. Data are collected, using an electronic data capture system, at screening, enrollment, and approximately quarterly throughout the duration of the patient’s participation in the registry. All patients provided informed consent. Enrollment is ongoing. The current analysis evaluated the percentage of patients with AML who had undergone molecular genetic testing recommended by NCCN guidelines (NPM1, FLT3-ITD, CEPBA, 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 academic vs community settings (76% [70/92] vs 62% [103/167], P=.018), normal vs acute myeloid leukemia (AML) types (77% [79/103] vs 59% [77/133], P=.006), age ≤65 vs >65 years (83% [85/103] vs 65% [108/181], P=0.003), and Medicare vs ACA (83% [65/78] vs 77% [77/99], P=.018). 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% (15/173) of patients, including 8% (6/77) 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 of patients did not undergo 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 c dependent adverse events (AE) in pts and identified the c of Q, but not its active metabolite, AC886, was a significant predictor of the Qc 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) age 18–55 years (yr) who provided informed consent were randomized 1:1:1 to receive K 200mg twice daily (BID), F 200mg BID, or placebo (P) BID on Days(D) 1-28. A single 30mg dose of Q was administered to all HS on D8. Plasma Q and AC886 conc were measured D8-28, using a validated liquid chromatography–tandem mass spectrometry method. PK parameters were determined using noncompartmental analysis. 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 subjects (31 per arm) and 88 received Q. 75% were male, median age 32 yr (18-53). Relative to Q+P, co-administration of Q+K or Q+F increased the geometric mean (GeoMean) Cmax of Q by 17% and 11%, and GeoMean AUC0-inf by 94% and 20%, respectively (Table 1 below). The GeoMean Cmax and AUC0-inf of AC886 were decreased by 60% and 15%, respectively, for Q+K, and were increased by 3% and 14%, respectively, for Q+F. Apparent clearance (CL/F) of Q was 50% lower and t1/2 of Q and AC886 were 46% and 96% longer, respectively in Q+K vs Q+F. CL/F of Q was 17% lower and t1/2 of Q and AC886 were 10% and 28% longer, respectively, in Q+F vs Q+P. AC886 is a minor component in circulation relative to Q (approximately 25%). An increase of 86% in simulated SS Q Cmax and 96% in SS Q AUC0-inf was predicted following repeat daily dosing of 30mg Q+K vs Q+F, while a modest decrease in AC886 exposure (<20%) was predicted. The most common adverse events were headache (7.5%) and diarrhea (5.4%), with the majority being Grade 1/2. There were no clinically significant hematological, 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cmax (ng/mL)</th>
<th>AUC0-24 (ng*hr/mL)</th>
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<tbody>
<tr>
<td>Q+K</td>
<td>17% increase</td>
<td>94% decrease</td>
</tr>
<tr>
<td>Q+F</td>
<td>11% increase</td>
<td>20% decrease</td>
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E926

**CLINICAL OUTCOMES OF CHILDHOOD ACUTE MEGAKARYOBlastic LEUKEMIA: THE CHILDREN CANCER HOSPITAL EGYPT 57357 EXPERIENCE**

**Background:** Acute megakaryoblastic leukemia is a rare subtype of pediatric AML occurring in both Down and non-Down syndrome patients. Down syndrome patients with M7 subtype have an excellent prognosis while non-Down syndrome patients have poor outcomes. Heterogenous cyto genetic abnormalities have been described with M7 AML and the impact of different prognostic factors on outcomes is yet to be determined.

**Aims:** To evaluate the prognostic significance of various cytogenetic abnormalities and minimal residual disease (MRD) by flow cytometry after induction I and correlate them with clinical outcomes of patients with acute megakaryoblastic leukemia.

**Methods:** We retrospectively analyzed the data of 80 non-Down syndrome patients diagnosed with M7 AML treated at CCHE between January 2007 through December 2016. Three treatment protocols were used.

**Results:** The median age at diagnosis was 1.7 years (range 0.2-15). The 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. Twenty two patients had MRD<0.1% after induction I. In the univariate analysis patients with MRD <0.1% post induction I had a better OS and EFS with a lower cumulative incidence of relapse however these findings did not reach a statistical significance.

**Summary/Conclusions:** Acute megakaryoblastic leukemia in non-Down 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**

C. Niederwieser1, C. Rohde1, H. Servé2, W. Berde3, G. Eiringer4, S. Göllner1, L. Müller1, C. Müller-Tidow1

**Background:** Methylation of DNA contributes to the development of cancer and correlates with clinical outcome. The epigenetic alteration of DNA methylation is recognized as an important mechanism in leukemia pathogenesis. Understanding the DNA methylation patterns in hematological malignancies provides insights into the epigenetic events in leukemia. The identification of resistance associated DNA methylation changes is important for treatment optimization and novel therapy development.
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, and those paired samples of day 0 and day 15 frequently clustering together as well. This led us to refine blast independent analyses. We excluded methylation changes correlating with the percentage of blasts (p=0.14, exploratory regression among blast change and median methylation change change day 0 to day 15, each), since these are likely to reflect the increased lymphocyte counts in patients with the 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 treatment.

Results: In the Azacytidine plus Chemotherapy treated group, a total of 389 differentially methylated regions (DMRs), most of which were single CpGs, were identified, 176 of which were hypermethylated and 213 hypomethylated. The most highly represented hypermethylated loci were INSM1 (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 ARID5B (p=1e-15, 2.82% of 213 DMRs), 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-45, 6.10%), while those most commonly hypomethylated were RUNX1 (p=1e-24, 28.34% of 1429 DMRs), TCF4 (p=1e-21, 8.40%) and SNAI3 (p=1e-17, 1.05%). Median overall survival did not differ between the two treatment groups, with 153 days for chemotherapy and 143 days for the combination chemotherapy patients.

Summary/Conclusions: Methylation changes associated with azacitidine and chemotherapy of refractory patients were particularly found in genes previously associated with cancer and AML. DNA hypermethylation was more common after chemotherapy alone. This finding suggests that DNA hypermethylation of specific loci may be associated with therapy resistance. Hence, the methylation levels were detected from the most resistant cells. Of note, upon Azacitidine treatment more hypomethylated loci were observed. This potentially indicates DNA hypomethylation in vivo.

E928
OVER-EXPRESSION OF ZEB2-AS1 LNCRNA PREDICTS POOR OUTCOMES IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA
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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 AML cases.

Methods: Relative quantitative real-time PCR analysis was employed for detecting levels of ZEB2-AS1. SYBR Green RT-PCR was performed, followed by obtaining relative threshold cycle normalized to reference GAPDH gene. Cell migration, invasion, proliferation and apoptosis tests were used to analyze biological phenotypes of AML cells after knocking down ZEB2-AS1 IncRNA by small interfering RNAs.

Results: Results showed that expression of ZEB2-AS1 IncRNA was prominently high and closely correlated with adverse clinical outcomes in AML patients, based on either modified MRC or ELN risk stratification system. Univariate analyses indicated that patients with higher expression of ZEB2-AS1 IncRNA had significant shorter 3-year overall survival (OS) (0% vs 68.2%, p=0.036) and disease-free survival (DFS) (25.0% vs 69.8%, p=0.039). In addition, Patients with higher expression of ZEB2-AS1 IncRNA had significant lower complete remission (CR) rate in response to induction chemotherapy (75.0% vs 27.3%, p=0.031). In patients with low levels of ZEB2-AS1 IncRNA, patients treated by allogeneic hematopoietic stem cell transplantation had significant longer OS (3-year OS, 75.8% vs 28.6%, p=0.037) and DFS (3-year DFS, 81.8% vs 26.8%, p=0.049) compared to that of chemotherapy.

Summary/Conclusions: Moreover, knockdown of ZEB2-AS1 IncRNA could effectively inhibit invasion and migration in AML cells, which was closely associated with down-regulation of ZEB2 and up-regulation of E-cadherin. Collectively, although independent prognostic value for survivals was not rigorously determined, ZEB2-AS1 IncRNA may serve as candidate to improve conventional risk stratification system and contribute to evaluating therapeutic responses.

RESULTS

CONSOLIDATION IS SAFE AND WELL TOLERATED IN OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMIA
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Background: AML in the elderly is more susceptible to treatment failure. Treatment related mortality in elderly patients with AML is decreasing over time, and receiving chemotherapy of adequate intensity is important in treating AML in these patients. The optimal induction and consolidation approach for patients in this age group is yet to be established, however data from the HOVON group has demonstrated the benefit of anthracycline intensification during induction in patients aged 60-65 years, while locally the Australian AML12 study demonstrated the value of anthracycline intensification during consolidation in younger adults. We have implemented a novel combination of intensified anthracycline in combination with infusional cytarabine (AraC) during induction and in combination with intermediate-dose AraC during consolidation.

Aims: To demonstrate the safety and tolerability and provide preliminary efficacy evidence for anthracycline intensification during induction and consolidation in older adults with Acute Myeloid Leukaemia.

Methods: A retrospective pilot study was done on 76 consecutive patients above the age of 55 years with newly diagnosed AML between January 2010 to June 2016 at Alfred Hospital, Melbourne, Australia. All received the 7+3 induction regime (AraC continuous infusion at dose of 100mg/m2/day on days 1 to 7, and idarubicin at a dose of 12mg/m2/day on days 1 to 3), with a planned consolidation with AraC (AraC 100mg/m2 twice daily Day 1, 3, 5, and idarubicin 12mg/m2/day Day 1-2). Outcomes were assessed according the Cheson criteria with cytogenetic risk assessed by the refined Grimwade MRC criteria.

Table 1

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<tr>
<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Median age of patients received</td>
<td>62 years (range 55.4-70.6 years)</td>
</tr>
<tr>
<td>Median overall survival after 7+3 induction</td>
<td>109 days (range 6-1988)</td>
</tr>
<tr>
<td>Median relapse-free survival</td>
<td>314 days (range 6-4197)</td>
</tr>
<tr>
<td>Median event-free survival</td>
<td>590 days (range 6-1996)</td>
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Results: 76 patients, with a median age of 62 years (range 55.4-70.6 years) received the 7+3 induction with a median overall survival of 590 (range 6-1996) days and overall response rate was 52 patients (68.4%). The event-free survival median is 109 days (range 6-1988) and the relapse-free survival median is 314 days (range 6-4197). There were 7 treatment-related deaths (11.8%) within 30 days following 7+3 induction. Of 41 patients who attained complete morphological remission after induction, 29 patients (70.7%) received the planned IDAC+2 consolidation with 17 (41.5%) receiving two consolidation cycles. Of those not receiving IDAC+2, 10 patients (24.4%) received an alternative consolidation regimen and 2 patients (4.9%) did not receive consolidation. Of those receiving IDAC+2 25 (86.2%) were intermediate cytogenetic risk and 3 (10.7%) were unfavorable. No treatment related deaths occurred following IDAC+2. 20 patients (26.3%) from the whole cohort received an allogeneic stem cell transplant (SCT), and 8 patients (27.6%) of those who received the IDAC+2 consolidation regime proceeded to an allogeneic SCT. In all IDAC+2
consolidation cycles, the median days to neutrophil recovery was 26 days (range 18-72), platelet recovery 32 days (range 17-75), and the ICU admission rate was 12.8% (range 2-10 days). 18 patients (62.1%) receiving IDAC+2 consolidation suffered disease relapse. For patients receiving IDAC+2 consolidation the median OS was 727 days (range 113-1614 days) with an EFS of 388 days (range 109-1614 days). For patients aged 60-65 years the remission rate and overall survival were similar to those published by Lowenberg et al.

Summary/Conclusions: Anthracycline intensification was well tolerated with low treatment related mortality and rates of ICU admission along with acceptable time to count recovery. In patients aged 60-65 outcomes were similar to published data with high-dose daunorubicin. Despite this intensive post-remission therapy approach rates of disease relapse were high highlighting the need for novel therapeutic approaches in this patient group.

E930

PROGNOSTIC IMPACT OF IDH1 AND IDH2 MUTATIONS IN LOW AND INTERMEDIATE RISK AML: A MULTICENTER RETROSPECTIVE STUDY

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Background: Mutations in the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes are common in acute myeloid leukemia (AML) but, although investigated in several studies, their prognostic significance still remains controversial.

Aims: To evaluate the prevalence and prognostic impact of IDH1 and IDH2 mutations in adult AML patients with low and intermediate-1 and 2 risk (European Leukemia Net, ELN 2013).

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 12.66x10⁹/L in IDH wild-type, and 24.71 x10⁹/L in IDH mutated. Absolute neutrophil count was 3.1x10⁹/L in IDH wild-type and 0.9x10⁹/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 and IDH status.

Summary/Conclusions: Our study demonstrated that IDH1 and IDH2 mutations showed no impact on OS and DFS.
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=2050) 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=0.66-0.81)). In patients <60 years, never-married patients were less likely to receive intensive therapy (adjusted OR 0.43 (CI=0.19-0.99)) than married patients. In patients <70 years achieving CR, the chance of alloHCT was reduced when living alone (11.8%, adjusted OR 0.47 (CI=0.28-0.78), versus 19.0% in patients living with someone). In divorced/widowed, 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=1.08-1.57), 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 affecting overall survival. Increased focus on what drives treatment decisions in patients lacking social support is important to improve survival in these patients.

**E933**

**TREATMENT OF MOLECULAR RELAPSE IN ACUTE MYELOID LEUKEMIA WITH DIRECTED THERAPY REDUCES TOXICITY OF SALVAGE TREATMENT AND IMPROVES DISEASE CLEARANCE**

F. Guolo1,*, P. Minetto1, M. Clavio1, D. Guardo1, E. Covilli1, N. Colomb1, F. Balerini2, M. Miglioli1, R.M. Lemoi1, E. Gobbi1
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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 allo stem cell transplantation. The important 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 leukemic associated immunophenotype (LAIP) changes during chemotherapy and post-therapy disease burden, assessed by NPM levels, was explained by social support rather than by differences in income and occupation.

**Aim:** To investigate the announcement and clearance of MRD reduction and LAIP changes in de novo AML during chemotherapy and compare the results with clinical outcomes.

**Methods:** In clinical prospective study since March 2016 till February 2017 50 patients (pts) were included in the study. 14 pts by this moment completed basic chemotherapy (ChT) courses: 7+3 and 2 consolidation. Among them favorable cytogenetics was in 4pts (t(16;21)-1, 16q22-1, 8-21-2 pts), intermediate-7 (6-with normal cytotype, t(17;22)), poor-3 (complex karyotype-2, 11q23-1pt). Bone marrow samples were studied in standardized panel with most common antibodies by 6-color MFC (BD FACSCanto II, USA) before the treatment, after 1st and 2nd cycles of induction and after 2nd consolidation. Among patients that MRD >0 was assumed as MRD positivity. Besides MRD status we also explored LAIP changes in patients with CR 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 complete morphological remission (CMR) after the second course and during ChT one of them gained CD56 and CD13, 2nd lost CD65 and CD11b, 3rd – gained CD65, 4th gained CD11b after 2nd ChT, the last one didn’t change LAIP. We detected relapse in 3 pts from this group and one – with increasing MRD after 4th course and cytopenic syndrome. We may suggest that LAIP changes during ChT reflect selection of more chemoresistant leukemia clone, followed by subsequent relapse.

**Summary/Conclusions:** 1. The most favorable group of patients consisted of MRD negative pts after 1st course. LAIP changes are common in pts with less favorable prognosis.

**E934**

**MINIMAL RESIDUAL DISEASE AND LAIP CHANGES BY FLOW CYTOMETRY IN DE NOVO ACUTE MYELOID LEUKEMIA DURING CHEMOTHERAPY AND CLINICAL OUTCOMES**

T. Lobanova1,1, I. Galtseva1, Y. Dayvova1, N. Kapranov2, V. Troitskaya1, E. Parovichnikova1
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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 leukemic associated immunophenotype (LAIP) changes during chemotherapy and post-therapy disease burden, assessed by NPM levels, was explained by social support rather than by differences in income and occupation.

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**Summary/Conclusions:** 1. The most favorable group of patients 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 IN CR1**

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1Leukemia, MD Anderson Cancer Center, Houston, United States

**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 leukemic associated immunophenotype (LAIP) changes during chemotherapy and post-therapy disease burden, assessed by NPM levels, was explained by social support rather than by differences in income and occupation.

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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 ≥100. Patients were treated continuously with lenalidomide 10mg PO daily on D1-28 of a 28 day cycle up to 24 cycles. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. After cycle 1, stepwise dose escalations were allowed to 20mg daily in pts who were tolerating their dose and have presence of minimal residual or morphologically detectable disease.

Results: A total of 14 patients have been enrolled with a median age of 57.5 years (range, 23-67). All pts were in CR at the time of enrollment, with 12 pts (86%) in CR1 and 2 (14%) in CR2. Baseline pt characteristics are outlined in Table 1. AML-related mutations detected at start of therapy included: CEBPA(n=5), NPM1 (3), FLT3 (3), IDH2 (2), NRAS (2), DNMT3a (2), and 1 each of JAK2, TET2, and EZH2. High risk features at the time of enrollment were as follows (some are overlapping): S (36%) with history of prior myeloid neoplasm or therapy related AML, 4 (29%) persist 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 89%, 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-hematotoxic 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>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54 (18-74)</td>
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<tr>
<td>WBC (×10^9/L)</td>
<td>4.6 (2.3-9)</td>
</tr>
<tr>
<td>Platelets (×10^12/L)</td>
<td>119 (71-213)</td>
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<tr>
<td>LDH</td>
<td>128 (59-2128)</td>
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<tr>
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<tr>
<td>Crude creatinine</td>
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</tbody>
</table>

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 and two cycles of intermediate dose cytarabine (IDAC) in patients ≥60 years. Competing risk cumulative incidence estimates were calculated for NRM and relapse. Early relapse and NRM were defined as relapse/NRM before completion of postremission therapy.

Results: Median age was 52 (18-74) years. Median follow up time was 481 (31-3384) days. Early relapse rate (RR) and NRM were 11.01% and 5.29%, respectively. Median OS after early relapse was 128 days. Presence of FLT3/ITD mutation and high body mass index were associated with increased risk of early relapse on multivariate analysis (HR 14.88, 95%CI 3.24-68.43 and 2.34, 95%CI 1.3-4.2, respectively). Age increased risk of early NRM (HR 5.13, 95%CI 1.5-17.58 for age 55-55 years). 76% of patients completed therapy: 42% received allogeneic HCT in CR1, 21% completed three cycles of HIDAC and 13% completed two cycles of IDAC. 3-year OS and RFS of the whole cohort were 53.68% and 40.26%, respectively. OS was 67% in a group of patients who completed HIDAC, 34% in IDAC group and 64% in HCT group (p=0.28469). Cumulative incidence of NRM and RR 3 years after completion of therapy were 23% and 20% after HCT, 7.1% and 51% after HIDAC and 16.8% and 66.4% after IDAC, respectively, differences among groups were significant (p=0.00947 and p<0.00001). HCT reduced the risk of relapse in comparison to chemotherapy (HR 0.51, 95%CI 0.3-0.85). RFS was adversely influenced by concomitant FLT3/ITD/NPM1 mutation (HR 2.17, 95%CI 1.06-4.45). Increasing age had negative effect on OS (HR 1.65, 95%CI 1.13-2.42 for age 55-35 years). After HCT, HLA mismatch and TBI based myeloablative conditioning were associated with increased NRM (HR 6.32 (95%CI 1.89-21.14) and 6 (95%CI 1.86-19.2), respectively) in comparison to transplantation from HLA matched donors and 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. 15-25800A. All rights reserved.
LONG TERM FOLLOW UP OF PATIENTS OVER 60 YEARS TREATED WITH INTENSIVE CHEMOTHERAPY FOR ACUTE MYELOID LEUKAEMIA 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 following treatment in 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 26 years ago.

Aims: To characterize the long term outcome of elderly AML and high risk MDS patients treated with intensive chemotherapy after the usual 2-5 year follow up period.

Methods: We treated 160 patients aged 60 years or more suffering from high risk MDS and AML with intensive chemotherapy regimen between 1991 and 2004. None of the patients underwent allogeneic stem cell transplantation afterwards. We now performed a follow up of the surviving patients 10 years after publication of the initial study.

Results: In the initial study median survival from the start of induction therapy was 9.5 months (10 days to 157 months), with the median survival from diagnosis of 14 months (1 day to 157 months). At publication of the study in the year 2007, 20 patients were still alive, 18 of them presented with a low risk karyotype. 13 of these patients were in complete remission and 7 patients had relapsed. Since then 11 of the 13 patients who were in CR relapsed and died of their leukemia. One patient died of other causes and only one patient is still alive and well, currently at the age of 84. This patient initially presented with a normal karyotype, too. As a result the rate of long term survivors 5 years after treatment is 5.6% only.

Summary/Conclusions: Long term follow up data of elderly patients treated for AML and MDS with intensive chemotherapy is scarce. Our data show, that induction chemotherapy not followed by allogeneic stem cell transplantation does not result in a meaningful improvement of outcome. In addition, morbidity and lack of quality of life has to be taken into account. More data and studies on this subject are urgently needed in an aging population. In our population of 160 treated patients, 158 died of their leukaemia, only one patient died of another cause and only one single patient is still alive and well over a decade later.

E938

FLAG-IDA FOR RELapsed/REFractory ACUTE MYELOLeUKAEMIA: A SINGLE CENTRE 5-YEAR STUDY

Background: The treatment of relapsed/refractory Acute Myeloid Leukaemia (AML) remains a formidable challenge as the therapeutic options are limited. The regimen most commonly used in this setting, FLAG-Ida (Fludarabine, cytarabine, G-CSF and 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 its efficacy in an elderly patient profile in the routine clinical setting.

Methods: We conducted a retrospective chart review of patients treated with FLAG/FLAG-ida chemotherapy regimen for relapsed or refractory acute myeloid leukemia (including secondary AML) between 2011 and 2016 in a large tertiary hospital. Patients treated with FLAG/FLAG-ida as first line therapy were excluded.

Results: Of 80 patients eligible for the analysis, 95% (95% CI 2.4-6.4), 13.4 months, from first line therapy was associated with an inferior survival following FLAG-ida compared to best supportive care or intensive chemotherapy. (Int J Hematol 2014; 100: 141-151).

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 LEUKAEMIA WHO WERE TREATED WITH DECITABINE

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 cytogenetics 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 arise 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 best supportive care or intensive chemotherapy. (Int J Hematol 2014; 100: 141-151).

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. According to 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 cytogenetic 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.

Summary/Conclusions: Compared to intensive treatment, decitabine is widely accepted as the treatment options for elderly AML patients. However, the efficacy has yet been evaluated in Asian population where difference of clinical manifestation or cytogenetics had been noted. In this study, we present a single tertiary centre experience in the use of this regimen with a view to identifying predictive factors for survival following FLAG-ida chemotherapy. The secondary aim of this project was to assess its efficacy in an elderly patient profile in the routine clinical setting. 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 arise in elderly patients.

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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. According to 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 cytogenetic 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.
E940

**DRUG-DRUG INTERACTION POTENTIAL OF GILTERITINIB IN HEALTHY SUBJECTS AND PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA**


San Francisco, 3Mayo Clinic, Rochester, 4University of Pennsylvania-Abramson Comprehensive Cancer Center of Northwestern University, Chicago, 6Astellas Pharma

*Background:* Gilteritinib (ASP2215), a highly selective FLT3/AXL tyrosine kinase inhibitor with activity against both FLT3-ITD and FLT3-D835 mutations, was administered as a single 20-mg dose on Day 8. Additionally, the potential effects of CYP3A4 inhibitors (itraconazole [ITZ] and fluconazole [FLZ]) 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 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) was assessed in a cohort of patients with R/R AML (n=9) in the Phase 1/2 CHRYSSALIS study (NCT020144585). 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, FLZ) CYP3A4 inhibitors were compared with those for patients not using CYP3A4 inhibitors.

*Results:* In healthy subjects, gilteritinib exposure (expressed as Cmax and AUC0-24) was higher (2-fold increase) in subjects who were coadministered gilteritinib with a strong CYP3A4 inhibitor (ITZ) than in subjects who were administered gilteritinib alone. Coadministration of gilteritinib with RIF, a strong CYP3A4 inducer, resulted in an approximate 70% decrease in gilteritinib exposure in healthy adult subjects (Figure 1). In patients with R/R AML, midazolam exposure was approximately 10% higher when administered with gilteritinib compared to midazolam alone as reflected by the geometric mean ratio and 90% confidence intervals of midazolam Cmax (111.64%; 69.54%–179.25%) and AUC0-24 (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 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.

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**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**

E. Zappone1, L. Aprile1, M. Defina1, G. Papini1, V. Cardi1, G. Bartalucci1, C. Zuanelli Brambilla1, S. Ciofini1, M. Bocchia1

1Hematology Unit, University of Siena, Siena, Italy

*Background:* For decades no effective new drugs or better anthracyclin cytara- bin combinations other than the standard 3 + 7 regimen have been available for AML induction treatment. Fludarabine-based regimens have shown good efficacy in relapsed patients but raised concern about toxicity in the induction setting (Burnett JCO 2013, PMID 23940227) a modified regimen has shown better tolerance and good results in patients younger than 60 years (ys) (Guolo AJH 2016, PMID 27084986)

*Methods:* Patients were treated with the FLAIE or FLAI regimen followed by Idarubicin or Fludarabine. Aracytin, Idarubicin or with or without Etoposide: FLAIE up to 65ys or FLAI up to 75ys.

*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 (34/136), 9% of patients (12/136) had secondary AML 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 when patients are well selected, low CMR groups had a significant difference in terms of survival and factors significantly affecting OS were age below 50ys p<0.0001; good/intermediate CMR risk p<0.0002; intensive consolidation with Allo or Auto transplant p<0.0001 compared to chemotherapy alone. The mean probability of Leukemia free survival (LFS) was 88 months (median not reached). Patients above 50ys and patients with karyotype profile (NPM1/FLT3-ITD) the median probability of OS and LFS were 16.4 and 23.4 months respectively, this compares favorably with many published results. Chen Medicine 2016 PMID: 27472687 reported a median OS of 10.3 months in a large cohort of patients of similar age treated with intensive induction. Moreover we did not found a significant difference between the 50-59ys and 60-75-ys 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 very feasible and manageble in patients beyond age of 60ys a difficult population to treat with a curative intention mainly because of concern of high toxicity of intensive induction regimens and higher incidence of poor risk prognostic factors.

E942
OVEREXPRESSSION OF SOX4 CORRELATES WITH POOR PROGNOSIS OF ACUTE MYELOID LEUKEMIA
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Background: The SOX4 belongs to the SOX (Sry-related high-mobility group box) family and has been characterized as a transcription factor. Over the past decade, multiple functions of SOX4 have been unveiled, and the protein is now known to play important roles in embryonic development, cell fate decision, and cellular differentiation. Overexpression and amplification of SOX4 have been implicated in various cancers and are correlated with poor prognosis. In mouse models, previous studies demonstrated that the upregulation of Sox4 can be induced by and then cooperate with the aberrant expression of AML-1, ETO, NUP98-DDX10, and MLN-RARA; the overexpression of HOXA9, CREB, and Evl1, 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 as a direct target of C/EBPα. C/EBPα is known to inhibit the self-renewal of leukemic cells and to restore cellular differentiation. The overexpression of Sox4 that results from C/EBPα inactivation contributes to the development of a type of leukemia that is characterized by a distinct leukemia-initiating cell (LIC) phenotype. This 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 and non-parametric 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=50) and high expression group (score 3, n=50), respectively. The variance in clinical manifestations of AML did not show significant differences in terms of SOX4 expression. However, AML patients with low SOX4 expression tended to have favorable-risk cytogenetic (P=0.0866). We did not observe significant differences between the high and low expression groups in terms of age, gender, hemogams, 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 relapse rates compared to low SOX4 expression (19/36, 52.8% vs 13/49, 26.5%, P=0.028). Furthermore, with a median follow-up period of 46.7 months (range: 0.3 to 70.9 months), SOX4 expression was associated with overall survival (OS) and disease-free survival (DFS) in all patients with de novo AML (P=0.008 and P=0.013, respectively), patients with non-M3 subtypes (P=0.001 and P=0.011, respectively), patients with intermediate-risk cytogenetics, (P=0.001 and P=0.005 respectively), or even in those with normal karyotype profile (P=0.022 and P=0.111, respectively). In multivariate analysis, high SOX4 expression was found to be an independent poor prognostic factor for OS (RR 1.924, 95% CI 1.020-3.628, P=0.043) irrespective 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 THROMBOPOETIN AS A ADJUNCT AFTER INTENSIVE CONSOLIDATION CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIA
X.-H. Sui1,*, Y. Li1, X. Wang2
1Hematology department of Shandong provincial hospital affiliated to Shandong University. 2Hematology department ofShandong provincial hospital affiliated to Shandong University, Shandong University school of medicine, Jinan, China

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 20×10^9/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 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 50×10^9/L after induction therapy, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0–3. Patients with FAB M3 (acute promagranulocytic leukemia) and FAB M7 (acute myelomonoblastic 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 intermediated-dose arabinosyl cytosine (Ara-C), et al. When the platelet count was less than 50×10^9/L, patients in study group received 15000u/day of rhTPO (trade name: TPIAO) administration subcutaneously and patients in control group not received rhTPO therapy. The administration of rhTPO continued until the platelet count was more than 100×10^9/L or for the maximum of 21 days. Statistical analysis: Baseline characteristics were compared using chi-squared test or independent 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: The rhTPO was no statistically significant difference 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 study group were less than those in the control group, but there were no significant differences of statistical status between the patients. Platelet recovery: 1. rhTPO might reduce the duration of platelet count less than or equal to 20×10^9/L and 30×10^9/L after chemotherapy. 2. rhTPO could increase the maxi
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.

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<th>Study Group</th>
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<td>Minimum platelet count</td>
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Summary/Conclusions: rhTPO, administered as dose of 15000u/day when platelet count less than or equal to 50×10^9/L, might improve the recovery of thrombocytopenia of patients with acute myeloid leukemia in CR after consolidation chemotherapy. While there was no significant difference between study group and control group, there was a decreasing trend of platelet 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 (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 21%, p=0.07), and those receiving LD-AraC compared to BSC had significantly improved OS (median: 9.6 vs 3.4 months, p<0.001). In this same study, while OS was longer with HMA than LD-AraC, this difference was not significant (median OS 16.1 vs 9.6 months; 3-year OS 22% vs 12%, respectively; p=0.1). Two studies assessed the efficacy of AZA vs moderate-IC, LD-AraC, or palliative therapy, alone or in combination. AZA had a significantly better survival rate vs LD-AraC in poor prognosis patients (p=0.015). Furthermore, 1-year survival was higher for AZA-treated patients (67.8%) compared to those not treated with AZA (36.9%) (p=0.004). The efficacy of AZA relative to other conventional care regimens (CCRs) including BSC, LD-AraC, or standard IC was also examined in a randomized clinical trial (n=488). Median OS at 1-year was significantly higher for AZA relative to CCR (10.4 vs 6.5 months). Results also showed that 1-year median OS was higher with AZA than CCR in all cytogenetic risk groups, normal risk (14.1 vs 10.0), intermediate risk (13.0 vs 10.1), and high risk (6.4 vs 3.2), respectively.

Summary/Conclusions: Among older AML patients, IC tended to be associated with improved OS compared with other CCRs. However, evidence from this review indicates that AZA could be an alternative treatment option for older AML patients, whether fit or unfit for IC.

E945

SYSTEMATIC REVIEW OF HEALTH STATE UTILITY VALUES FOR ECONOMIC EVALUATION OF ACUTE MYELOID LEUKEMIA

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Background: Cost-utility analyses undertaken to inform decision making regarding acute myeloid leukemia (AML) require a set of health state utility values (HSUVs) so that the time AML patients spend in different health states can be aggregated into quality-adjusted life-years (QALY).

Aims: This study reviews AML-related HSUVs that could be used in economic evaluation and assesses their advantages and disadvantages with respect to valuation methods used and AML clinical pathways.

Methods: Embase, MEDLINE, Cochrane database, and conference abstracts (ASCO, ESMO and ASH) were systematically searched from Jan 2000 through Nov 2016 for relevant studies that reported quality of life (QOL) and HSUV in AML. Identified relevant EORTC Quality of Life Core Questionnaire QLQ-C30 values were mapped to HDUV using previously published algorithm by Crott, et al. 2010. HSUV for induction, consolidation, complete remission (CR), relapse, stem cell therapy (SCT) treatment, SCT recovery and CR post SCT were identified.

Results: Ten relevant studies were identified. Six were cost effectiveness analyses utilizing HSUVs for calculation of Quality Adjusted Life years (QALY), one effectiveness analysis (incremental QALY). Two QOL studies reporting specific AML utilities (either collected or mapped from QLQ-C30). An additional study reported QOL for patients undergoing SCT. Since no study reported HSUV for relapse, values from study of secondary AML patients who failed prior treatment for Myelodysplastic Syndrome, were used. While multiple HSUVs were available, prioritized clinical trials were used to identify HSUV are presented in Figure. AML treatment (both induction, consolidation and SCT) was associated with decreased HSUV, while post-treatment CR lead to increased HSUV.
Summary/Conclusions: This interim analysis of the use of decitabine in real life showed a superiorimposable 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 compared to decitabine 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 CHRYSVANTHEMI EFFECTIVELY DEPLETES PLASMA GLUTAMINE, HAS CLINICAL ACTIVITY, AND IS WELL TOLERATED IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Asparaginase-induced glutamine (Gln) depletion demonstrates anti-leukemic activity in preclinical studies of AML. We hypothesized that administration of asparaginase Erwinia chrysanthemi (Erwinaze) would lead to effective plasma Gln reduction and may be a feasible therapeutic approach for AML, because myeloblasts may be addicted to Gln.

Aims: The primary aim was to determine the dose of Erwinaze inducing plasma Gln levels <120 μmol/L with an acceptable safety profile, 48 hours (h) after the first intravenous (IV) dose and before each subsequent dose administered thrice weekly for 2 weeks in patients (pts) with relapsed or refractory (R/R) AML.

Methods: This was a phase 1, single-arm, pharmacokinetic investigator-initiated trial (NCT02283190, funded by Jazz Pharmaceuticals), with a 3+3 design with dose de-escalation/escalation rules that incorporate both safety and biochemical activity (nadir plasma Gln levels) of IV 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 pts experienced DLT, the trial was to be terminated. To evaluate Gln reduction ability of Erwinaze, the dose could be increased based on 48h trough plasma Gln in cohorts of 3, 6, or 9 pts per dose level. Correlative studies measured plasma Gln, glutamate (Glu) and asparagine (Asn) levels, plasma asparaginase activity and plasma and urine 2-hydroxylgluturate (2-HG) levels.

Results: Five pts were enrolled on study. Enrolment 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 CML, 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 IU/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 ≥0.1 IU/mL was achieved in all pts at 48h trough, but in 3 pts it decreased to zero on day 8 (72h trough). Median trough plasma Gln, Asn and peak Glu levels (μmol/L) at 28h 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 2.44 (1.78-3.42) (p<0.001). One achieved partial remission (PR) and one achieved hematologic improvement (HI) after 6 doses of single agent Erwinaze. Both pts had plasma Gln levels <65 μmol/L on days 5, 10 and 12. Off study, after completion of Erwinaze, they were treated with azacitidine. Both pts are still alive in complete remission (CR) and CR with incomplete count recovery (CRi) 13.3 and 13.4 months after the on-study date. Plasma and urine 2-HG levels did not change significantly. The 3 pts with IDH mutations tended to have higher plasma 2-HG levels (p=0.10).
Summary/Conclusions: To the best of our knowledge, this is the first clinical report demonstrating that an asparaginase product is capable of not only decreasing plasma Glu 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 0.01 (0.32-2.54), 1.00 (0.45-5.73), 1.00 (0.19-2.54), 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 was 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 far as the impact that SOX expression positive status has 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=0.017; p=0.017, respectively). Although all of the SOX+ patients had shorter overall survival (OS) time compared to SOX- 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 significantly 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. As for the exact function of these genes in the pathogenesis of AML is not yet known, our preliminary results show that their overexpression can have prominent prognostic significance in AML patients and therefore should be the subject of further investigation.

E949
ACUTE ANTHRACYCLINE INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background: Chemotherapeutic agents are associated with a wide range of cardiotoxic adverse effects. Anthracyclines and related drugs are some of the most implicated agents, with a well-recognized potential for the development of cardiomyopathy and heart failure. Chronic anthracycline induced cardiotoxicity can lead to cardiomyopathy, which may develop several years after treatment. Acute and subacute anthracycline induced cardiotoxicity is considered relatively uncommon, described mostly in patients treated for solid tumors or lymphomas. While anthracycline based regimens have been used to induce remission in newly diagnosed patients with acute myeloid leukemia (AML) for more than four decades, relatively little is known about the acute cardiotoxic effect of anthracyclines in this setting. Since many of these patients were candidates for hematopoietic stem cell transplantation (HSCT), an intensive intervention usually reserved for fit patients, even transient decrease in cardiac function might render them ineligible for this intervention, or might increase their transplant related morbidity.

Aims: To study the short-term outcomes of anthracycline exposure on cardiac function in patients with AML who are candidates for allogeneic HSCT. Because current AML-induction regimes use anthracyclines (most commonly daunorubicin) at a relatively high dose between 45 and 90mg/m2/day for three consecutive days, we hypothesized that the incidence of post-induction cardiac injury in these patients is 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, 6.1%) and 90mg/m2/day (N=15, 27%).

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 higher rates of cardiac function deterioration (odds ratio: 4.1, 95%, confidence Interval: 1.06 to 15.7). Patients with cardiovascular risk factors and male patients tended to develop cardiotoxicity at higher rates, whereas age, white blood cell counts at diagnosis and AML type (de novo vs. secondary) had no impact on cardiotoxicity. The decrease in cardiac function was temporary in 10.9% of the patients (N=6) with subsequent normalization of left ventricular EF in those patients.

| Table 1. |
AN INTEGER WEIGHTED GENOMIC MUTATION SCORING (IWGMS) USING THE TRUSIGHT MYELOID SEQUENCING PANEL SHOWS HIGHER MORTALITY IN PATIENTS WITH INTERMEDIATE RISK ACUTE MYELOID LEUKAEMIA: A RETROSPECTIVE STUDY

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Background: AML is currently classified by European LeukemiaNet into favorable, unfavorable, and intermediate prognosis based on cytogenetic aberrations. Although favorable and unfavorable categories have good prognostic values, the intermediate category encompasses the majority of patients and offers unclear prognosis. The development of Cancer Genome Atlas (TCGA) opens new windows for the incorporation of next generation sequencing (NGS) into cytogenetics to enhance prognostic risk stratification. However, few studies explore the combination of cytogenetics and NGS in prognostic predictions.

Aims: Here we have developed a system of Integer Weights for the Genomic Mutation Score (IWGMS) for a quantifiable stratification of the prognostic risks associated with a combination of cytogenic aberrations and genomic mutations. Our next step is validating the scoring system through its application to data obtained from other institutions.

Methods: Patient data at Houston Methodist Hospital was queried from the Methodist Environment for Translational Enhancement and Outcomes Research (METEOR), a clinical data warehouse that integrates research data-bases and national registries. The diagnosis of AML was queried along with patient demographics, cytogenetics, NGS and OS. The resultants patients were divided into three categories based on their MRC cytogenetic risks: favorable, intermediate, and poor. Using the TruSight Myeloid Sequencing Panel (Illumina), mutations in 54 genes associated with myeloid disorders were tested in NGS. A scoring system was developed that assigned each of the nine TCGA mutation categories (Transcription- Factor Mutation, Nucleophosmin (NPM1), Tumor Suppressor Genes, DNA-Methylation related genes, Signaling Genes, Chromatin Modifying Genes, Myeloid Transcription Factor Genes, Cohesion complex Genes and Spliceosome-complex genes) a score between -2 (good risk) and +2 (poor risk). The IWGMS for each patient was calculated by the sum of the individual mutation scores. A IWGMS score greater than 3 was considered significant as a poor prognostic factor. Statistical analysis was done using Chi-Square, Mann Whitney U test and multivariate logistic regression analysis. Data from other institutions will be analyzed in a similar fashion for the confirmatory portion of the project.

Results: A hundred of the 1200 AML patients met the criteria for having both cytogenetic and NGS data availability. The two-year mortality rates were 43%, 52%, and 51% respectively for the favorable, intermediate, and poor cytogenetic groups. In the intermediate cytogenetic group, high IWGMS score (>3) was associated with higher mortality when compared to low IWGMS score (80% vs 44%, p=0.045, Fig 1). A look at the gene mutation distribution in the intermediate risk cytogenetic group also showed a general correlation between known favorable gene mutations with low IWGMS scores and unfavorable ones with high IWGMS scores. We thus hypothesize the IWGMS scoring system can be utilized to divide patients into higher and lower mortality subgroups based on a combination of cytogenetic and genetic mutations. We expect similar results with data from other institutions.

Summary/Conclusions: Most studies in current literature focuses on the individual contributions of cytogenetic aberrations or genetic mutations to risk stratification and treatments risk stratification and treatment response. However, prognosis varies widely in the heterogeneous, intermediate cytogenetic class, where 60% of the AML patients belongs. We propose a systematic approach that correlates 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.

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SUCCESSFUL IDENTIFICATION OF SPECIFIC AMINO ACID-DEPENDENCE IN ADULT T-CELL LEUKAEMIA / 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 tried 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 lympocytes of healthy volunteers were also examined. Finally, the effectiveness of amino acid restriction was evaluated in vivo by xenotransplantation of ATL cells into NOD 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 effect a significant change in the proportion of normal CD4+ populations, such as Treg, naive, central memory, effector memory, and effector T-cells. Moreover, 4-week restriction of valine succeeded in eradicating ATL cells in vitro and no recurrence was observed after refilling valine although 2-weeks restriction was insufficient for extermination. 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.

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 the growth of ATL cells was significantly inhibited by dietary restriction of valine. Massive lymphoma cells, which are known to be resistant to antibody therapy, were also vulnerable to the valine restriction. There were no severe complications such as anemia, thrombocytopenia, and organ damages which are often seen in chemotherapy recipients. These data demonstrate that valine restriction may potentially provide a new option for leukemia/lymphoma therapy.

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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 (rs699947), -2489 C/T (rs1005230) -117 C/G (rs1570360), -634 G/C (rs2010963), -460 C/T (rs833081), 936 C/T (rs3025039) and VEGFR2 -2710 G/A (7667289) and -604 T/C (rs2071559) SNPs determine higher production, transcriptional activity or binding efficiency of VEGF/VEGFR2.
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 through DNA of peripheral blood by real-time polymerase chain reaction using a Taqman SNP Genotyping Assay. Replicates were performed in 10% of the reactions, achieving 100% of concordance. Chi-Square test, Fisher’s Exact test, and multivariate analysis, using the logistic regression model, served to assess associations between genotypes and clinical aspects. Kaplan-Meier analysis was used to evaluate the effect of clinical features and genotypes on the progression free survival (PFS) of BMI at baseline, assessed by means of BMB and PET/CT. Results were considered statistically significant when P<0.05.

Results: Concerning clinical features, the frequency of the wild-type VEGF -634GG genotype was more common in stage II or IV patients. The wild-type VEGFR2 -604TT genotype was more common in high intermediate and high international prognostic index (IPI) patients. Concerning response rate, patients with the wild-type VEGF 936CC genotype was associated with higher complete response (CR). These patients had a high proportion 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% and 66%, respectively. At 60 months of follow-up, patients with the variant VEGF 1154 A allele and 936 T allele 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 A SNP was associated with PFS and OS: patients with the variant VEGF 1154 A allele had 1.88 and 1.83 more chances of having an event.

Summary/Conclusions: Our data present, for the first time, preliminary evidence that inherited abnormalities in AG pathway, related to the VEGF -1154A/G, -634GG and 936CT/C, and VEGFR2 -604TT/C, influence clinical 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 BM and/or PET/CT performed simultaneously as part of the routine pre-therapy staging for newly diagnosed DLBCL. Patients who had not received 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 variables that are independent of analysis were 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 detainees and 46 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 (113 females and 92 males). Twenty-one of these patients (10.3%) had BMI on BMB, 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 p<0.150, were: female gender; IPH3, abnormally elevated B2-microglobulin levels, PET/CT-BMI(+) and BMB-BMI(+). In multivariate analysis only two factors were retained: age +IPI (HR: 3.15, 95%CI 1.92-5.1; p<0.001). The four patients with a worse survival. In this cohort, BMI by PET/CT could not independently predict a shorter PFS and/or OS.

Conclusion: Our data indicate 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 on progression-free survival (PFS) and overall survival (OS) in newly diagnosed DLBCL patients.

Results: Eighty-nine patients (male, 52) were enrolled. The median age was 65 years (range, 19-88). 37 patients (41.6%) were classified as high-intermediate (HI) or high risk according to National Comprehensive Cancer Network International Prognostic Index (NCCN-IPI). CD11b+CX3CR1+ monocytes were enumerated in the study. The results are summarized in the table. The expression level of CD11b+CX3CR1+ cells was 3.31% (range, 0.21 to 21.66%) in PB and 3.09% (range, 0.20-20.01%) in BM. Patients were categorized into high (PB- or BM-CD11b+CX3CR1+ cells >median) and low (<median) groups. High PB-CD11b+CX3CR1+ cell group was significantly associated with unfavorable features, including age >60 years, advanced stage, elevated serum LDH level, and extranodal involvement (P<0.05), which were clinical factors associated with higher risk NCCN-IPI (P<0.004). However, BM-CD11b+CX3CR1+ cells were not associated with clinical variables. With a median follow-up of...
27.7 months (IQR, 14.6–46.1), 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-variate 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, HI or high risk NCCN-IPI was an only independent prognostic factor for reduced OS (hazard ratio, 4.41; 95% confidence interval, 1.17-16.59) in the multivariate analysis. In subgroup analysis according to the NCCN-IPI, 3-year OS of high PB-CD11b+CX3CR1+ monocytes was significantly inferior to that of low group (34.0% vs 77.9%; P=0.026) in B-NHL, but not in T/NK-NHL. 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 differential patients with high risk for early death and are associated with risk stratification by the NCCN-IPI, possibility of potential therapeutic target in DLBCL.

E955
RARE NON-HODGKIN LYMPHOMAS (R-NHLs) IN CHILDREN: THE AIEOP EXPERIENCE
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Background: Clinical management of pediatric rare non-Hodgkin lymphomas (r-NHL) (<1 case/1million) 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 watch 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 1 case. Patients who presented LDH >500 UI were 18. B-NHLs accounted for approximately 49% (33 pts) of the entire population analyzed. BM-NHL accounted for other 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-NHLs; peripheral T-cell lymphoma (PTCL) n.o.s., mycosis fungoides (MF), subcutaneous panniculitis T-cell lymphoma (SPTCL) and lymphomatoid papulosis (LP) amongst T-NHLs; histiocytic sarcoma (HS) amongst “others” category. A similar proportion for both B and T/NK NHL underwent either W&S approach only or active treatment (AT): 45% and 55% were W&S and AT approach, respectively. Patients in “others” category were almost completely treated (71%). Therapy was based on AIEOP B-, T/NK-NHLs and ALCCL protocols and / or immunotherapy. Surgical resection has been performed in case of localized disease B-NHLs only, followed by a W&S strategy, with 100% 3-yr OS. It has been seen that B-NHLs have a more favorable prognosis and very few events (development of resistance to therapy, relapse, secondary malignancy, death). Amongst T/NK-NHLs-related events, death remained the most common event. In case of B-NHL, the most common cause of relapses; as for the category “others”, no relative preponderance has been registered for any of the above-mentioned events. The 3-year OS has shown to be significantly higher for B-NHLs than for T/NK-NHL (94% vs 69%, p-value 0.024), as illustrated in Figure 1. Regarding the treatment, the 3-year OS was 100% for the patients underwent a W&S approach whereas 75% for treated patients (p-value 0.037). FLs show favourable clinical course and outcome, limited stage at diagnosis. Differently from adults, pFLs have a higher 3-years OS with respect to that of other histological pediatric NHLs subtypes (100% vs 75%, p-value 0.049).

Figure 1.

Summary/Conclusions: The incidence of AIEOP pediatric rNHLs is in line with the literature. In case of localized disease, a W&S approach was successfully applied; of these, the T/NK rNHLs being most often registered and with best prognosis are the cutaneous lymphomas (i.e. LyP, MF). Patients’ prognosis varies greatly depending on the histological subtype. The better survival was observed in the B-NHLs compared to other categories. An international collaboration is warranted, in order to create new guidelines or protocols for an appropriate management of pediatric rNHLs.

E956
PRIMARY ANALYSIS OF THE EFFECT OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF 110 CASES OF T CELL LYMPHOMA
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Background: T cell lymphoma(T-NHL) is a rare and heterogeneous group of lymphoid malignancies with mostly poor outcome with conventional treatment. Recent studies have suggested that Hematopoietic stem cell transplantation(HSCT) has a better curative effect and is superior to traditional chemotherapy.

Aims: To investigate the effect of HSCT in the treatment of T cell lymphoma.

Methods: The clinical data of 110 patients with T cell lymphoma treated by HSCT from January 2006 to August 2016 in our center were retrospectively analyzed.

Results: (1)110 T-NHL patients, 70 males and 40 females, aged 7-64 years (median age 26 years). Disease subtypes: 35 cases of T-cell lymphoblastic lymphoma(T-LBL), 23 cases of NK / T cell lymphoma(NK/TCL), 24 cases of peripheral T-cell lymphoma (PTCL, NOS), 24 cases of peripheral ALCL, 1 case of hepatosplenic T cell lymphoma(HSTCL) and 1 case of histiocytic lymphoma(HLCL). Transplantation type: 56 cases of autologous hematopoietic stem cell transplantation (auto-HSCT), 54 cases of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The follow-up was ended in December 2016, the duration of follow-up ranged from 2 to 130 months (median follow-up time was 22 months). (2)56/110 patients with auto-HSCT, 3 year overall survival (OS) and disease-free survival (DFS) were 76.5% and 60.9%, respectively. (3)54/110 patients with allo-HSCT, 3 year DFS and OS of allo-HSCT were 61.7% and 58.9%, respectively. (4)36/56 patients with CR1 status before auto-HSCT, 3 year OS and EFS were 87.3% and 68.7% respectively. (5)56/110 patients with non-CR1 status before auto-HSCT, 3 year OS and EFS were 60.6% and 40.2%. 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). 20/56 patients with non-CR1 status before auto-HSCT, the 3 year OS and EFS were 47.6% 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 CENTER EXPERIENCE

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Background: Mantle cell lymphoma (MCL) is considered an incurable disease with an historical median overall survival around 3-4 years with short progres-

sion free survival (PFS) periods. Regimens that include high dose cytotoxicare 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 review our expe-
rience 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-Hyper-
CVAD. Clinical characteristics at diagnosis of these 33 patients were: MiP ratio: 26/7 (78.8%/21.2%), median age: 63 y.o. (limits: 40-73), ECOG 0-1; 26 (86.7%), Ann Arbor stage III-IV 28/31 (90.3%), MiPi score: low risk: 5 (16.7%), interme-
diate 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 longer considered for ASCT, 1 rejected, 1 unknown cause. Conditioning regimen was BEAM/LACE in 18 (90%) patients and cyclophosphamide-TBI in 2 (10%). One patient died 10 days after infusion for sepsis. With a median follow-up of 35 months (1-315) months, the median PFS was 73.0 (95%IC 38.2-107.8) months (8.08 years) for the whole group, 114 (47.3-180.7) months for the transplanted patients vs 21 (3.1-38.9) months (1.8 years) for the not transplanted group. The median OS was 123 (31.9-214.1) months, median OS was not reached for transplanted group vs 31.0 (7.5-54.6) months for not transplanted.

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.

E958 THE FREQUENCY OF INCIDENTAL MALIGNANCIES DETECTED BY PET/CT SCANS IN PATIENTS WITH LYMPHOMA AND THE ASSOCIATED CLINICAL IMPLICATIONS

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Background: PET/CT imaging has a well-established role in the investigation of malignant lymphoma. Given the widespread clinical applications, unexpected findings are occasionally identified. Whilst there is substantial information pertaining to additional primary cancers identified on PET/CT in patients with solid organ malignancy, there is a relative paucity of data in patients with lymphoma.

Aims: The primary aim was to identify the frequency of incidental second malignancies identified by PET/CT imaging in patients with lymphoma. Qualitative data related to histological diagnosis and staging, interruptions or obstacles to lym-
phoma 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-allowed indications were included. All PET/CT reports suggest 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 implica-
tions 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 lym-
phoma. Patients aged 17 to 96 were included in the study, with a median age of 62 years. Of the 259 patients included (M=155; F =104), 55 patients had a diagnosis of Hodgkin’s lymphoma and 204 patients a diagnosis of Non-
Hodgkin’s lymphoma. A total of 33 out of 295 patients with a diagnosis of malign-
nant lymphoma had PET/CT findings suspicious for an underlying second malignancy (12.7%). Of the 33 patients, 19 underwent further invasive inves-
tigation, with a total of 8 patients having a biopsy proven pathological 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 sec-
ond malignancies were early stage and gastrointestinal in origin. Further ret-
rospective as well as prospective data may assist in the establishment of guide-
lines, to address a standardized diagnostic approach to investigating incidental lesions discovered on PET/CT imaging that are suggestive of a second malignance.
and was approved by the institutional review boards of all participating institutes.

Results: Among the 465 DLBCL cases, karyotypic analyses by G-banding were performed on biopsied tumor specimens before the start of treatment in 181 patients. Among the 181 patients, metaphase spreads were available for G-banding in 120 patients. Neither overall survival (OS) nor progression-free survival (PFS) was statistically significantly different between the patients with available metaphase and no available metaphase spreads. Based on the result of G-banding, we next divided the 120 patients with available metaphase spreads into two groups, i.e., patients with karyotypic abnormalities accompanied by ≥2 subclones and patients with 0-1 subclones. We found that the presence of ≥2 subclones was significantly associated with poor OS (3 year OS rates of patients with ≥2 subclones and 0-1 subclones were 67.6% and 82.8%, respectively (p=0.035), and tended to associate with a shorter PFS. Among the 120 patients with available metaphase spreads, the R-IPI-defined high-risk patients and KPI-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., DLBCL with 0-1 subclones and with ≥2 subclones, because the OS was the most markedly different between these two groups. In our study, more subclones were associated with poor prognosis, suggesting the significance of karyotypic evolution in DLBCL. In conclusion, our study suggests that more advanced cytogenetic clonal evolution underlies the development of high-risk disease feature in DLBCL.

E960
REGIMENT INTENSIFICATION MAY IMPROVE OUTCOMES IN PATIENTS WITH HIGHER RISK HUMAN IMMUNODEFICIENCY VIRUS (HIV) RELATED AGGRESSIVE B-CELL LYMPHOMAS
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Background: Despite effective combination antiretroviral therapy for HIV, there remains an increased incidence of HIV related B-cell Non-Hodgkin lymphomas (NHL). The introduction of early antiviral therapy and effective chemotherapy have led to improved outcomes overall. Regimen intensification (RI) in HIV associated B-cell NHLs has shown improved survival, especially in the rituximab era (Barta et al, Blood 2013).

Aims: To examine the effect of RI on the overall survival (OS) and progression free survival (PFS) compared to CHOP based chemotherapy according standard risk stratification.

Methods: Patients with HIV associated aggressive B-cell NHL were identified between 2001- 2015 at Moffitt Cancer Center. Patients with primary central nervous system lymphoma, T-cell NHL and indolent NHLs were excluded. Patients received R-CHOP or intensive chemotherapy (IC) including DA-EPOCH, hyperCVAD or CODOX/IVAC as initial treatment. Data collected included patient demographics, disease baseline characteristics, CD4 count, HIV viral load, treatment regimen, response, and outcomes including relapse and OS. The IPI score was calculated, and patients were divided into two groups: lower risk group (low and intermediate IPI risk) and higher risk group (high-intermediate and high).

Results: A total of 83 patients were included. The M:F ratio was 9.4. Median age was 65 years (y) (range 25-86). Two thirds of patients were Caucasian. The median time from HIV to NHL diagnosis was 29 months (90 – 284). Eighty two percent presented with stage III/IV disease. Bulky disease was present in 27%, elevated LDH in 66%, and CD4 count<100/mL at diagnosis in 22% patients. Fifty percent of patients were on HAART therapy at time of lymphoma diagnosis (PFDS). Chemotherapy regimens included: R-CHOP (n=30, 36%), CHOP (n=12, 15%), DA-EPOCH-R (n=27, 33%), DA-EPOCH (n=1, 1%), hyperCVAD (n=11, 13%) and CODOX/IVAC (n=2, 2%). The median follow up was 2.7 y (95% CI 1.2-3.0 y). The median OS and PFS for the whole cohort was 5.9 and 1.4 y, respectively. The median OS was 4 y (95% CI 1.5-6.5) for patients who received CHOP, the median OS for those with lower risk disease was NR (p=0.05). For patients who received IC, the median PFS was NR among lower and 1.4 y higher risk groups (p=0.34).

Summary/Conclusions: The IPI score remains prognostic in HIV related B-cell NHLs. There was a trend for improved OS and PFS using IC regimens. CHOP treatment remained associated with worse outcome among higher risk patients while IC regimens may overcome the higher risk features based on the IPI.

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 expression 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 extranodal sites seemed to have higher expression of BCL2 and higher DNH 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 DNH, patients with ENKTL could be divided into three significantly different risk groups for PFS and OS (3-year PFS rate for DNH of 0, 1, and 2 was 60%, 41%, and 21%, respectively, p=0.008; 3-year OS rate for DNH of 0, 1, and 2 was 79%, 49%, and 33%, respectively, p=0.015). In multivariate survival analysis, it was found that DNH was an independent prognostic factor for both PFS and OS (p=0.006 and 0.011, respectively).

Summary/Conclusions: Our study demonstrated that DNH 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 120 months (range 1-584), 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 1, MALT in 2, peripheral T-cell lymphoma, not otherwise specified in 3, Hodgkin lymphoma in 4, and LPD in 4. EBER in situ was examined in 30 patients with 7 patients. The median observation period in the surviving patients was 34 months (range 3-119). The 2-year progression-free survival (PFS) and overall survival (OS) rates for all 40 patients were 69% and 79%, respectively. A total of 18 patients (45%) had spontaneous regression after ISD withdrawal. The median time from ISD withdrawal to spontaneous regression was 4 months (range 1-13). Among the 18 patients with spontaneous regression, 3 relapsed. Of the 22 patients without spontaneous regression, 20 subsequently underwent chemotherapy; 18 underwent CHOP/CHOP-like ± rituximab, and 2 underwent other regimens. In total, 7 patients died: all died from lymphoma progression. Compared to those without spontaneous regression, patients with spontaneous regression had clinical stages I-II (P=0.021), performance status of 1-2 (P=0.028), normal levels of LDH (P=0.026), and lower levels of sIL-2R (P=0.005). The ROC curve analysis showed the appropriate cut-off of sIL-2R levels to be 2400 U/mL for predicting spontaneous regression (AUC, 0.74; sensitivity, 0.81; specificity, 0.67). On multivariate analysis, only advanced stages of OI-LPD was associated with spontaneous regression (odds ratio, 0.03; 95% CI, 0.002-0.39; P=0.007). Thirteen patients with DLBCL who did not have spontaneous regression received CHOP/CHOP like ± Ritux. The CR rates, and 2-year PFS and OS of these 13 patients were 38%, 68.3%, and 82.1%, respectively.

Summary/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.
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 tumor progression difficult to establish and hinder the use of new personalized treatments in clinical practice. In this context, there is currently a necessity to define new biomarkers enabling a better definition of DLBCL subtypes, prognosis evaluation and an overview of the resistance to chemotherapeutics. We decided here to focus on circulating microRNAs that are found in all biological fluids. This accessibility makes them good candidates for biomarkers studies.

Aims: This research aims at studying microRNAs found in plasma from DLBCL patients and at investigating their potential as biomarkers of survival in these patients. For this purpose, a plasma biobank was created with samples from DLBCL patients at different times of their treatment. This follow-up of microRNAs level during the course of treatment is particularly innovative in this study.

Methods: A plasma biobank from DLBCL patients was set up at the Centre Hospitalier Universitaire (CHU) UCL Namur Yvoir, Belgium (ethical agreement number BO39201419613). 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 course (C2 and C4) and at the remission review (Cf). In the case of an autograft, a sample was taken at the post-graft review (Cpgr). The first step of this study was the selection of the microRNAs that will be quantified in all the samples of the biobank and that would potentially be used as biomarkers. To this end, a quantification of 377 microRNAs was performed by TaqMan® Low Density Array on the plasma samples of two selected DLBCL patients and one healthy donor with no history of cancer. These DLBCL patients were selected based on their highly different response to treatment. One of them obtained a complete remission after a 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 progress, 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-19b, miR-19a, let-7e and miR-21 have been selected in this study and are currently quantified 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.
OVERALL SURVIVAL IN YOUNGER NEWLY DIAGNOSED HIGH-RISK INTENSIFIED TREATMENT REGIMENS IMPROVE EVENT-FREE AND OVERALL SURVIVAL IN PATIENTS WITH DLBCL. More prognostic information can be obtained from sarcopenia, which is related to intolerance to R-CHOP therapy and to worse survival.

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/m^2 i.v.) followed, in the case of persisting evidence of CR or PR, by a maintenance treatment with monthly courses of lenalidomide (10mg/m^2, 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. PD-ESR was performed for the assessment of therapy response 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=71) and 21% (n=22) respectively. Two patients died due to multiple organ failure and disease progression after 1 and 8 months from diagnosis, respectively. In our frail 80-year-old patient cohort, the sequential treatment strategy was well-tolerated. After R-BENDA cycles, grade II infectious disease was observed in 2/11 patients (18%) and DNA-CMV reactivation was detected in other 2 additional patients (18%). However, 2 out of five patients who started maintenance lenalidomide treatment discontinued therapy for 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 S.-I. Go1, M.-J. Park2, G.-W. Lee1 1Internal Medicine, 2Radiology, Gyeongsang National University School of Medicine, Jinju, Korea, Republic Of

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 patients.

Aims: In this study, given the uncertainty about the optimal SM1 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 SMl (PM-SMI) who were treated with standard 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-pectoral muscles (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.
**Summary/Conclusions:** Our data suggests that the addition of etoposide to R-CHOP and increase in dose-intensity improves EFS and OS of younger patients with newly diagnosed high-risk B-LCL. R-CHOEP/14 and DA-R-EPOCH seem to be similarly effective in this setting.

**E970**

**HIGH COMORBIDITY INDEX ALONG WITH HIGH NCCN-IPI STRONGLY INFLUENCE SURVIVAL OF Diffuse Large B CELL LYMPHOMA PATIENTS: SERBIAN Lymphoma Group EXPERIENCE**

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**Background:** A few studies have validated the prognostic significance of the NCCN International Prognostic Index (NCCN-IPI) so far. However, some patients with low risk according to NCCN-IPI have poor survival, and thus clinical parameters, that might better characterized patients within risk groups, need to be explored.

**Aims:** The aim of this study was to evaluate prognostic significance of current indexes such as International Prognostic Index (IPI), NCCN-IPI, and the influence of comorbidities on the overall survival (OS) of patients with newly diagnosed diffuse large B cell lymphoma (DLBCL).

**Methods:** A total of 708 patients (383 males/345 females) with the median age of 58 years (range 18-89) were included in the study. Majority of patients received R-CHOP (Rituximab, Cyclophosphamide, Doxorubicine, Vincristine, Prednisone) protocol, 652 (92.1%), while 29 (4.1%) received R-EPOCH (Rituximab, Etoposide, Cyclophosphamide, Doxorubicine, Vincristine, Prednisone), and 27 (3.8%) received R-CVP (Rituximab, Cyclophosphamide, Vincristine, Prednisone).

**Results:** According to the Ann Arbor classification, stage I and II had 332 patients (46.9%), while stage III and IV had 376 patients (53.1%). Bulky disease was present in 201 patients (28.4%), and B symptoms in 437 patients (61.7%). Majority of patients had cardiovascular disorders (223, 31.5%), endocrinological (63, 8.9%), neuropathological (132, 18.6%), and other (13, 1.8%). According to IPI, low, low intermediate, high intermediate and high risk had 332 patients (46.9%), 174 (24.6%), 132 (18.6%), and 70 (9.5%), respectively, while according to NCCN-IPI, 133 (19.6%) patients had low risk, 335 (47.3%) low intermediate, 198 (28.0%) high intermediate, and 36 (5.1%) high risk. Overall treatment response (ORR) was achieved in 615 patients (86.9%). Disease relapse was confirmed in 116/615 patients (18.9%). The patients with B symptoms (Log Rank=18.50, p<0.0001) and bulky disease (Log Rank=14.79, p=0.0001) had inferior OS compared to those without B symptoms or bulky disease. All parameters incorporated in IPI, as well as in NCCN-IPI, were significantly associated with OS (p<0.01). Moreover, the patients with at least one comorbidity had inferior OS (Log Rank=5.41, p=0.20), as well as those with high CCI ≥2 (Log Rank =7.59, p=0.006). Regarding OS, IPI (Log Rank=97.36, p<0.0001), and NCCN-IPI (Log Rank=102.29, p<0.0001) confirmed its prognostic significance. Furthermore, the patients with high CCI had significantly inferior median OS in the high risk group according to IPI (19 months vs 37 months), and NCCN-IPI (12 months vs 19 months).

**Summary/Conclusions:** NCCN-IPI represents useful prognostic index in DLBCL patients. It can be used in the clinical practice to better describe patients within risk groups, compared to IPI. Moreover, comorbidities contribute to inferior survival through frailty, drug dose reduction and poorer tolerability.

**E971**

**SUBSTITUTING DOxorubicin WITH ETOPoside IN r-CHOP RESULTS IN A REGIMEN WITH SIMILAR EFFICACY FOR TREATMENT OF NEWLY DIAGNOSED ELDERLY PATIENTS WITH B-LARGE CELL LYMPHOMA (B-LCL)**

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**Background:** R-CHOP is standard front-line treatment for B-LCL. However, anthracycline-induced cardiac toxicity limits its use in elderly and patients with preexisting heart disease. R-CEOP, in which doxorubicin is substituted with etoposide, has been suggested as a potential solution of this problem, but reports on the efficacy of this regimen vary substantially, especially in patients with non-GC DLBCL. We have been using this regimen regularly for front-line treatment of patients with B-LCL and preexisting heart disease and present here our experience.

**Methods:** We performed a retrospective analysis of all newly diagnosed B-LCL patients treated with R-CEOP at our centre from 2011 to 2016 and compared them to patients 60 years or older treated during the same period with R-CHOOP, the standard regimen used at our centre for non-frail elderly without significant cardiac comorbidities. The dose of etoposide in R-CEOP was 50 mg/m2 iv or 100 mg/m2 orally daily for 3 days. Both regimens were given every 3 weeks for 6-8 cycles. Patients with initial bulky disease or in PR after systemic treatment were irradiated.

**Results:** 31 patients, 15 male and 16 female, received R-CEOP and 48, 25 male and 23 female, R-CHOOP. Patients in the former group were older (median age 77 y, range 58-87 vs median age 66 y, range 60-83), had more often low performance status (81% vs 31%) and advanced disease (84% vs 54% stage 3 and 4) resulting in a significantly higher proportion of patients with IPI 3-5 (74% vs 40%, p=0.019). Proportions of patients with increased LDH were similar between the groups. There were no significant differences in frequency of grade 3-4 toxicity between the regimens; 48% of patients in both groups required emergency hospitalization; thrombocytopenia or anemia occurred in 16% of R-CEOP and 23% R-CHOOP treated patients, infections in 32% and 31% and cardiovascular events in 16% and 21%. However, 7 patients (23%) in the R-CEOP group died during treatment due to adverse effects in comparison to 4 (8%) in the R-CHOOP group. Efficacy was similar, 65% responded to R-CEOP and 79% to R-CHOOP. After a median follow-up of survivors of 27 mo, 3-y OS was 55% in the R-CEOP group and 52% in the R-CHOOP group, 3-y EFS was 50% and 50%, respectively (figure). Outcomes of patients with GC and non-GC DLBCL categorized according to Han’s algorithm were similar irrespective of treatment.

![Figure 1](image_url)

**Summary/Conclusions:** Long-term outcomes of newly diagnosed B-LCL patients treated with R-CEOP seem as good as those achieved with R-CHOOP irrespective of cell of origin. Observed differences in treatment-related mortality were most probably caused by differences in age, comorbidities and performance status. R-CEOP should be considered as a regimen of choice for B-LCL patients with cardiac contraindications for anthracycline treatment.
E972
POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: A SINGLE-CENTER CASE SERIES
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Background: Post-transplantation lymphoproliferative disease (PTLD) is a complication of both solid organ transplant (SOT) and haematopoietic cell transplant (HCT) and represent a very heterogeneous group.

Aims: The objective of this study is to evaluate the epidemiology, clinical features, characterization and therapeutic management of this disease.

Methods: We evaluated a total of 52 patients diagnosed between May 1995 and February 2017. We analyzed the following data: type of transplantation, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.

Results: Among the 52 patients, 31 were men (59.6%) and 21 women. PTLD after SOT were 45 (86.5%), of which 16 were after liver transplant (35.6%), 14 cardiac (31.1%), 9 pulmonary (20%), 4 renal (8.9%) 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 double umbilical cord blood and 1 autologous. Of the 52 PTLD, 48 were B lymphomas (92.3%), of which 26 were diffuse large B-cell lymphomas (DLBCL) (54.2%), 7 polymorphic (14.6%), 7 low-grade (14.6%), 4 Burkitt lymphomas (8.3%), 1 Hodgkin’s lymphoma (2.1%) and 1 T/NK lymphoma, 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, 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 Rituxin + Rituxin (17.8%). Three patients responded to reduction of immunosuppression (5.8%) and 3 did not receive any treatment for early death (5.8%). At the time of writing, 19 patients remain alive (36.5%) and 33 have died. The median survival of these patients was 19.5 months (0-198).

Summary/Conclusions: PTLD constitute a very heterogeneous group. Its appearance is much earlier in the HCT than in the SOT and, within this latter group, it is earlier after lung transplant and later after renal transplant. The most common type in our series is DLBCL. The majority are related to EBV, so post-transplant monitoring is essential, and its diagnosis is earlier than in EBV-.

Most low-grade lymphomas appear post-liver transplant, either in relation to viral infections 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 were identified from the Humedica, a large US electronic medical record database, between 01/01/08 and 07/31/15. DLBCL diagnosis was determined by the presence of ≥1 inpatient record or ≥2 outpatient records with DLBCL diagnosis codes; the first DLBCL record served as the index date. Following the index date, initiation of 1LT for DLBCL was required. For the assessment of the survival outcomes, patients were evaluated from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15).

Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using unadjusted Kaplan-Meier analyses.

Results: 1,436 newly diagnosed DLBCL patients who initiated 1LT met the patient selection criteria. 54.0% were male, and the mean age was 66.4 years (SD: 13.7) At baseline, 27.4% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (20.3%), chronic pulmonary disease (15.5%), and moderate to severe renal disease (9.5%). In 1LT, 92.1% of patients received combination therapy, with R-CHOP (63.5%) being the most common combination therapy. 7.9% of patients received monotherapy upfront, with rituximab (77.2%) being the most commonly used single agent. At 2 years following initiation of 1LT, the Kaplan-Meier OS and PFS were 79.2% and 67.3%, respectively. Median OS was not reached, and median PFS was 53.9 months (95% confidence interval: 45.2, 61.5). OS and PFS were also compared among patients receiving monotherapy vs combination therapy in unadjusted analysis. At 2 years, OS was 80.2% for patients receiving combination therapy vs 67.4% (P=0.0093) for patients receiving monotherapy. Also at 2 years, PFS was 68.3% for patients receiving combination therapy vs 55.1% (P=0.0051) for patients receiving monotherapy.

Summary/Conclusions: In this population of patients with newly diagnosed DLBCL receiving 1LT survival outcomes at 2 years were significantly improved for patients treated with combination therapy vs monotherapy. Future analysis will explore the differences in clinical characteristics of patients treated with monotherapy vs combination therapy in the 1LT setting.
**E974**

**AN EXPERIENCE WITH LONG ACTING FACTOR VII PROPHYLAXIS IN PAEDIATIC 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, 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 VIII prophylaxis can be a reasonable option for patients with haemophilia A in developing countries. It significantly reduces joint bleeds, school absenteeism, Joint scores significantly without risk of inhibitor formation and also improves all domains of quality of life.

**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 coagulopathy disorder resulted from mutations in the FVII gene (F7). The disease severity is not correlated with FVII levels but might be determined by molecular effects 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 hemarthrosis. There were eight different alterations identified. Four have been previously reported (c.1091G>A (p.R364Q), c.1238G>A (p.R413Q), c.1256C>T (p.T419M), and c.681G>T (IVS6+1T)). Four were novel (c.1192G>T (p.D398Y), c.1313G>T (p.G420V), c.291+2T>C (IVS3+2T>C), and IVS6-2A>G) that 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.D398Y), c.1313G>T (p.G420V), c.291+2T>C (IVS3+2T>C), and IVS6-2A>G) 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.
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 antidote. 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 (56.25%) patients with INR >8 and who have no signs of bleeding.

Summary/Conclusions: Our audit highlighted that there is less than 100% compliance in the recommended dose and route of vitamin K administration. A flowchart containing the guidelines will be designed to improve the management of high INR. To increase the awareness of this issue, teaching sessions for junior doctors and nursing staff are planned. A re-audit will be conducted once these steps are in place.

E979

NOVEL AND RECURRENT F7 MUTATIONS IN KOREAN PATIENTS WITH COAGULATION FACTOR VII DEFICIENCY

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Background: Coagulation factor VII deficiency is one of the 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 Cys89Gly 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 patients 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-Cy5.5, CD14-PerC7, CD64-APC, CD45-APC H7. We assessed the utility of single tube 5 color combination of FLAER-AF488, CD15-PE, CD15-PerCP-Cy5.5, CD64-APC, CD45-APC H7 for PNH screening and compared the results with the routinely used 6 color panel.

Figure 1.

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, hereditary anemia, 8-thalassemia, ITP; hepatitis C, multiple myeloma, Burkitt’s lymphoma. BM of 33 healthy donors was used as reference. FLAER was used for the reference values. MFC was performed according to International Leukemia-Net by 6-color cytometer BD FACSCanto II. We enumerated the proportion of CD34+ myeloid cells from CD45+ cells (normally <2%), the proportion of CD19+ (B-cell progenitors) from CD45+ cells (normally <5%), the expression of CD34, CD45, CD117, CD7, CD56 on CD34+ myeloblasts. Among granulocytes we analyzed: their proportion, granularity, CD14, CD64, CD45 expression and patterns CD16vsCD13, CD16vsCD11b, CD13vsCD11b. The final MFC conclusion was done by scale Ogata/Wells (van de Loosdrecht, 2013): A - does not correspond to MDS; B - reveals some features which commonly appears in MDS; C - results are consistent with MDS.

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 CD64 (53.6%), abnormal patterns (39.3%), low granularity (35.7%) in granulocytes; increased proportion (21.4%) and abnormal patterns (28.6%) in monocytes. 64.3% (n=9) patients with AA (CD157 was assessed as “A”, 21.4% (n=3) - “B” and 14.3% (n=2) - “C”. All “C” abnormal expression of CD117 and expression of CD56 on CD34+ cells were seen. AA patients with “B” and “C” showed increased expression of CD56, CD64 and decrease CD10 expression on granulocytes. Abnormal patterns were less common than in MDS patients. The increased proportion and CD56 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.).

Table 1.

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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1Madrid, Spain, June 22 – 25, 2017

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 (55-95% in PMPN) and were receiving eculizumab (ECU). Types of surgery were: 6 laparoscopic cholecystectomies, a transjugular intrahepatic portosystemic shunt, a distal splenorenal shunt, a laparoscopic Achilles allograft ligationoplasty, a gastrectomy, an emergency appendectomy, and 3 urologic interventions. Ten patients received ECU 900mg, while one patient surgery 6 received 1200mg since he had developed hemolysis at a previous surgical intervention (surgery 5). In two cases (patient G surgery 8; patient H surgery 11), an additional dose of ECU was administered before surgery. Patient H (surgery 11) had developed hemolysis at previous surgical interventions (surgery 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 6 and surgeries 10 and 11, respectively). The pre-surgical ECU dose was increased in surgery 6 (patient E) and an extra dose was administered in surgery 11 (patient H) and no hemolysis was observed. (See Table 1).

Summary/Conclusions: Our findings lead us to recommend to perform the intervention within 24 hours of the administration of Ecu in programmed surgery for which it is necessary to program the dose. While in urgent surgical interventions put a new dose on the day of the intervention independently of the previous dose. Also the normal ECU dose could be increased or an extra dose be administered in order to minimize the risk of hemolysis in high-risk patients or in those with a previous history of surgery-related hemolysis.

Figure 1.

Results: The median age of the study population was 49 years (range, 18-73 years) at eculizumab initiation and the median duration of eculizumab treatment was 34 months (range, 6-44 months). Median LDH fold x upper limit of normal was 7.29 (range 2.4-23.7) and GFI-deficient granulocytes was 92.8% (range, 15.7-100%) at the time of eculizumab treatment. PNH-related signs and symptoms were thromboembolism (TE, n=19), renal failure (n=20), pulmonary hypertension (n=5), and severe/recurrent abdominal pain requiring opioids (n=17). Of 46 total patients, 12 (26%) were classified as having PNH/AA and 34 with classic PNH. There were no substantial differences in laboratory findings, transfusion requirement, or clinical findings and symptoms 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 and the effect (hemoglobin above 10 g/dL) was sustained throughout 36 months in both groups. Transfusion-independence was achieved by 54.3% within the first 6 months of treatment and 86.4% by the last 36 months (83.3% in PNH/AA vs 87.5% in classic PNH). The mean number of RBC units transfused was significantly reduced from 8.5 units during the previous 6 months to 1.6 units for the first 6 months in total PNH patients (Fig). There were no significant differences in clinical outcomes (ie, LDH and transfusion unit per every 6 months) with eculizumab between the two groups. All TE (n=19) patients in whom 6 received concomitant anticoagulation therapy were resolved on the eculizumab; one classic PNH patient had recurrence of TE at the same site after discontinuation of anticoagulation therapy while on eculizumab. Among 9 patients who had baseline eGFR less than 60 ml/min/1.73m², 5 patients (56%) showed improvement of eGFR during the eculizumab treatment and 4 patients stabilized eGFR.
Summary/Conclusions: Clinical outcomes with eculizumab were significantly improved compared with the baseline in patients with both PNH/AA and classic PNH. This study demonstrated that eculizumab has a beneficial role in the management of patients with PNH/AA, similar to that of classic PNH, by inhibiting hemolysis and reducing transfusion requirements, thus resulting in the improvement of clinical signs and symptoms.

E985
DIAGNOSIS AND FOLLOW-UP OF THE CLONES OF PAROXYMAL NOCTURNAL HEMOGLOBINURIA BY FLOW CYTOMETRY
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a very rare chronic disease associated with a clonal expansion of one or several hematopoietic stem cells carrying acquired somatic mutations of PIG-A gene resulting in 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 2008 to January 2017. The PNH clone was investigated for bone marrow aspira- sion with or without haemolysis, regenerative hemolytic anemia with negative direct coombs test (DCT), myelodysplasia (MDS), unexplained cytopenia and thrombosis. The search for the PNH clone by FC is based on the analysis of the following monoclonal antibodies: Fcar and CD59 with gating on CD45 for neutrophils and CD14 for monocytes with gating on Glycophorin 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 the case of a very moderate or single-line deficit.

Results: Out of 234 cases analyzed, 201 cases (85%) showed absence of PNH clone and 33 cases (14%) had a PNH clone. There are 14 women and 19 men; Sex ratio (M/F) = 1.35, mean age = 42.27 years (17-73). Among patients that should be screened for positive PNH clone we have bone marrow failure: 25 positive (21.9%) of 114 cases screened, hemolytic anemia with negative direct coombs test: 4 positive/63 cases (6.34%), thrombosis: 2 positive/28 (7.14%), one negative case of AML2, myelodysplasia with 02 (11.2%) positive/18 cases and cytopenias: 0 positive/13 cases. The types of PNH were type II in 3 cases (9%), type III in 24 cases (72.9%) and mixed deficits in 6 cases (19%). The mean degree of CD14 deficiency was 29.4% (5-82) on red blood cells 48.21% (5-95) on neutrophil (N); the mean degree of Fcar was 55.33% (9-59) on lymphocytes (

Table 1. NK T cell level (%) in patients with AA in remission according to subgroups.

Summary/Conclusions: Thus, in patients with AA the decrease of NK-cell level was observed along with recovery of hemopoiesis in all the subgroup vari- ants. Previously we have shown that the decrease of NK-T cells accompanies the increase of TNF and IL-6, but there was no correlation between the degree of all subgroups.

E986
ASSOCIATION OF T-, B-, NK AND NKT CELLS WITH THE DURATION, COMPLETENESS AND OTHER CHARACTERISTICS OF REMISSION IN PATIENTS WITH APLASTIC ANEMIA
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Background: Immune-mediated dysregulation of hemopoiesis is the basis for pathogenesis of aplastic anemia (AA). Dysbalance of T cell subsets, especially Th1 and Th2, plays a significant role in the pathogenesis of this phenomenon. It is suggested, that NKT cells play an important role in the regulation 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) according to 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 tendency in their dynamics in all assigned subgroups, except for NK- and NKT cells in CR. NK cells in 10% of AA patients the level of NK-T cells in PB and BM exceeded normal level 1.8- and 2.2-fold, respectively. 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 patients with primary AA, NK-T cells decreased 2.8- and 1.9-fold, respectively, and in patients with remission ≥36 months it significantly decreased both in PB and BM (data presented in table 1).

Table 1. NK T cell level (%) in patients with AA in remission according to subgroups.

Summary/Conclusions: Thus, we have shown that the decrease of NK-T cells accompanies the increase of TNF and IL-6, but there was no correlation between the degree of all subgroups.

E987
A NOVEL DUAL-REAGENT SINGLE TUBE FLOW CYTOMETRY ASSAY TO SCREEN PAROXYMAL NOCTURNAL HEMOGLOBINURIA
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder resulting from loss of membrane-bound glycosylphosphatidyl inositol (GPI) anchor protein. The disease is characterized by heterogeneous clinical phenotypes including intravascular hemolysis, cytopenias, bone marrow hypoplasia and atypical site thrombosis. Screening guidelines recommend documentation of the lack of at least two GPI-liked anti- gens on at least two cell lineages. Alexa fluor 488 conjugated fluorescent Aerolysin (FLAER-AF488) has become a mandatory component in FCM based PNH assays.

Aims: We have analyzed the feasibility of a novel dual-reagent assay for screening PNH, by a single tube, cost-effective approach for PNH screening.

Methods: EDTA anti-coagulated peripheral blood of patients referred to department of Hematology with clinical suspicion of classical-PNH/ aplastic anemia, was tested with a single tube panel of FLAER-AF488/CD33APC. Simultaneously, the routine two tube flow cytometry assay (established sensitivity of 0.1%) for screening for PNH was performed. The panel (FLAER-AF488, CD59 and FLAER-AF488 and FLAER/CD33/CD14 for monococytes) 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 monococytes. The granulocytes and monococytes were considered positive and the respective clone sizes detected by both the strategies were compared.

Results: A total of 33 patients and 7 healthy controls were analyzed by both dual-reagent and conventional strategies. Among the thirty-three patients, twelve patients concurrently showed the presence of PNH clones by both methods. The rest twenty-one patients were negative for PNH clones in granulocytes and monococytes 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 3.78% (range, 0.2-18.2) and 3.60% (range, 0.1-18.6), respectively. The mean PNH clone sizes among the monocytes by dual-reactant 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=0.991, 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-reactant, 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 ELTROMBOPAG: EXPERIENCE OF A CENTER
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Background: Eltrombopag, a thrombopoietin receptor agonist, was approved in 2008 for the treatment of immune thrombocytopenic purpura. More recently, benefits demonstrated in the proliferation and maintenance of hematopoietic stem and progenitor cells (HSTC) led to its use and approval in the treatment of severe aplastic anemia (AA) refractory to immunosuppressive therapy.

Aims: In this report, we evaluated response to eltrombopag in patients with refractory AA and associated side effects.

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 allograft transplantation, treated with eltrombopag. 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 dexamethasone (1). Median duration of treatment with eltrombopag 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 had 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 hematological 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 eltrombopag was associated with hematologic response of one or more hematopoietic lineage, independence of side effects occurred. The median duration of treatment with eltrombopag 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 had 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 hematological abnormalities, that were rapidly resolved. One other patient had mild elevation of liver enzyme levels. No other relevant side effects occurred.

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Inhibiting Arginine Uptake in Chronic Lymphocytic Leukemia (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).

**Aim:** Determine whether CLL cells are capable of utilizing FFA for energy production. (B) Determine whether lipid metabolism in CLL is LPL dependent. (C) Determine why LPL is aberrantly expressed in CLL cells.

**Methods:** Peripheral blood (PB) and bone-marrow derived lymphocytes were obtained from previously untreated patients with CLL. Imaging of CLL cells was done by electron microscopy, and PB lymphocytes were stained for Oil red O. Confocal microscopy studies helped in determining the cellular localization of LPL. To study the capacity of CLL cells to utilize FFA we developed an assay that measured the oxygen concentration in the sera of cultured CLL cells prior to and after adding FFA. In addition we measured the oxygen consumption of CLL cells derived from ibrutinib-treated patients. We used immunofluorescence precipitation (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 cells with or without FFA for 20 hours to measure the consumption 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 media levels of CLL cell incubated without FFA. Remarkably, unlike cultured normal B cells, dO2 levels of cultured CLL cells did not change. 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 catalyzes the hydrolysis of lipids into FFA. Indeed, we detected LPL in the cytosol of CLL cells and in the cytoplasm of CLL cells. Furthermore, using small interfering RNA (siRNA) we knocked-down LPL mRNA levels and found that LPL-siRNA reduced the capacity of CLL cells to utilize FFA, suggesting that the lipid metabolism in CLL is LPL dependent. Because STAT3 is constitutively active in CLL cells, and because the LPL gene harbors STAT3 binding sites, we sought to determine whether STAT3 activates the LPL gene. Indeed, transfection of luciferase reporter gene constructs driven by LPL promoter fragments into MM1 cells revealed that STAT3 activates the LPL promoter. In addition, CHIP 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? L. Dong1, K. Williard-Clay2, S. Garaud2, H. Duvillier1, J.-N. Lodewyckx2, C. Solinas2, C. Gu-Trantien2, B. Stamatopoulou1, C. Sibile3, D. Bron1,7 1Clinical and Experimental Hematology, 2Molecular Immunology, 3Anatomopathology, INSTITUT JULES BORDET, ULB, Brussels, Belgium Background: Aging is characterized by a progressive decline in immune surveillance that favors tumor development in older patients. One mechanism used by malignant cells to escape immune surveillance is the upregulation of immune checkpoint inhibitors. Another process associated with aging is genetic or epigenetic modifications of tumor suppressor genes (TSGs).

Aim: To evaluate the correlation and potential role of BACH2 down-regulation and progression into a T cell lymphoproliferative disease, identifying the BACH2 gene as a candidate TSG. We thus examined the expression of specific transcription factors (BACH2 and PRDM1) and checkpoint inhibitors (PD-1 and PD-L1) in the major lymphocytes subsets for their potential role in immunosenescence.

Methods: Peripheral blood mononuclear cells were isolated from whole blood using Lymphoprep (Stemcell Technologies) density gradient centrifugation. Lymphocyte subsets (CD19+, CD3+CD4+; CD3+CD8+) were isolated for subsequent molecular analyses using the MACS Technology (Miltenyi), with the purity of each lymphocyte subpopulation between 95%-99%. PD-1 (PDCD1), PD-L1 (CD274), IL4, 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 lymphocytic leukemia (B-CLL) patients (median: 67yo). Healthy donors (HD) between the ages of 20 to 90 years subdivided into <50 yrs (median: 36yo) and >50 yrs (median: 65yo). BACH2 mRNA expression in the HD groups is significantly down-regulated in CD4+, CD8+ T cells and CD19+ B cells from the older HD group (P=0.0012; 0.0045 and 0.0067, respectively). BACH2 expression was further reduced in CD4+, CD8+ T cells and CD19+ B cells from CLL patients compared to HD well balanced for age (P=0.001; <0.0001 and 0.0043). PRDM1 mRNA expression was inversely correlated with BACH2 in CD4+, CD8+ T cells and CD19+ B cells (r=0.61; 0.71 and 0.85, respectively). Curiously, PRDM1 was – as expected – significantly upregulated in CD4+ T cells and CD8+ T cells (P=0.0034; P=0.0017) from B-CLL patients but not in their leukemic B. 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 down-regulation was correlated with increased IL-4 mRNA expression (r=0.67) but not IFNγ in CD4+ T cells. These observations suggest that BACH2 down-regulation in CD4+ T cells could enhance the expression of effector memory-related genes, particularly Th2 such as IL-4 and PRDM1, PD-1 mRNA expression was up-regulated in CD4+, CD8+ T cells (P=0.0153 and 0.0214) in the older HD group and also up-regulated in the T cells from B-CLL patients (P=0.0014 and 0.0023) when compared to age-matched HD population. High PD-L1 mRNA expression was correlated with increased age in HD B cells (P=0.04) with a further increase detected in HD PB (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 lymphocytes 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) I. Jiménez1,2, S. Bobillo3, P. Abrisquetta2, C. Palacios2, J. Carabia1, M.J. Terol1, M. Crespo1, F. Bosch1 1Experimental Hematology, 2Hematology, Vall de Hebron Institute of Oncology, Barcelona, 3Hematology, INCLIVA Biomedical Research Institute, Valencia, Spain Background: The different biological mechanisms leading the clinical progression of CLL from early stages are currently not fully elucidated. Different progression factors that decades a higher probability of progression, such as the presence of mutated IGHV genes, 6q deletion and positivity of CD38 and ZAP70. However, we are still not able to identify an important proportion of patients that eventually progress. Clinical progression from early stages to an advanced CLL is associated with a certainly reduced acquisition of molecular changes that are not able to explain the fifty percent of the CLL cases progressing. CLL cells are dependent on survival and proliferative signals from the microenvironment and are able to evade immune anti-tumoral responses using different mechanisms, which is a crucial feature for cancer development. T-cell dysfunction is one of the main sources of impaired anti-tumor immunity. In CLL, T cells show functional defects and have increased expression of the exhaustion markers PD1, CD244 and CD160 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 CD69 expression and the inhibitory receptors PD1, CD244, CD160, LA53, TIM3 and CTLA4. We also analyzed the expression of the transcription factors BACH2 and TIM3.

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=0.0223), PD-1 expression was significantly increased during progression in absolute numbers and up-regulated in CD8+ T cells (P=0.0161), 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 also observed that the percentages of CD38+ cells expressing CD244 and CD160 were higher at the time of progression, especially for CD244 (P=0.0078). Moreover, the co-expression of these markers with PD1 was found on CD8+ T cells and its percentage was increased during progression (P=0.0578). Among the differentiation subsets, the EM and EM CD45RA+ (TEMRA) CD8+ T cells expressed the highest percentages of CD244 and CD160. We did not observe changes in LA53, 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-bethi PD1int) or a terminal (Eomeshi PD1hi) phenotype. The percentage of TEMRA CD8+ T cells expressing Eomes and PD1 were significantly increased during progression (P=0.0186 and P=0.0286, respectively) whereas T-bet expression was more stable.

Summary/Conclusions: T cells from patients with progressed CLL show a more severe exhausted phenotype compared to diagnosis, which is characterized by an effector memory subset with higher expression and co-expression of PD1, CD244 and CD160, as well as higher levels of the transcription factor Eomes, indicating that the terminal exhausted phenotype (Eomeshi PD1hi) 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 STIMULATION IN CLL PATIENTS ON IBRUTINIB THERAPY S. Drennan1, G. Chioldi1, A. D’Avola1, P.W. Johnson3, L. Trentini2, G. Packham3, A.J. Steele3, F.K. Stevenson3, F. Corfoni1,4 1Haematology Oncology Group, Cancer Sciences Unit, University of Southampton, Southampton, United Kingdom, 2Cancer Sciences Unit, University of Southampton, 3Paola University School of Medicine and Department of Hematology and Clinical Immunology, Bambino Gesù Children’s Hospital, Rome, Italy, 4Haematology Branch, University of Padua, Padua, Italy, 3Cancer Sciences Unit, University of Southampton, 4Haematology Department, University Hospital Southampton NHS Trust, Southampton, United Kingdom Background: B cell receptor (BCR) signaling through surface IgM (slgM) 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 slgM,
but not of slgD, levels and signaling capacity. This is evident in the circulating CLL B-cells which are characterized by variably reduced slgM levels/signaling. The variability influences outcome and cases with relatively higher slgM levels/signaling capacity, but not slgD, have more rapid progression, likely due to a proliferative component.

Aims: The aim of this study was to investigate the effect of ibrutinib in vivo on the expression 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. Flow cytometry was performed on patients (REC: H228/02/01).

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 retrieval from antigen engagement at tissue sites. Also, the slgM levels correlated with increased anti-ιgM 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 in other BCR-associated molecules, appears consequent to lack of antigen encounter, likely due to inhibition of chemokine-mediated entry to tissue sites. In the later phases the circulating CLL cells will suffer lack of tissue derived proliferation 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 and idelisib 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-ERK1/2/2 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, idelisib 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 idelisib 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.
were observed while the combination with MK2206 was significantly more effective than either drug 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 activity 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 an 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 regulated by PKC, which may 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-1A AND ITS REGULATORY PATHWAYS AS A STRATEGY TO HAMPER LEUKEMIA-MICROENVIRONMENT INTERACTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: The CXCL12/CXCR4 axis has a fundamental role in the microenvironment-mediated protection of chronic lymphocytic leukemia (CLL) cells from spontaneous and drug-induced cell death. The binding of CXCL12 with CXCR4 activates multiple intracellular pathways, including RhoA- and Ras-dependent signaling. 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), HIF-1α inhibitor BAY87-2243 (1 μM), and PI3K inhibitor idelalisib (10 μM). RhoA and Ras activities were evaluated by an ELISA-based assay and by pull-down assay, respectively. ERK1-2, HIF-1α 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: In 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 toward spontaneous and fludarabine-induced apoptosis in CLL cells.

E1000
THE ROLE OF GENETIC-BASED PROGNOSTIC FACTORS IN PREDICTING MINIMAL RESIDUAL DISEASE NEGATIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND OFATUIMAB
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Background: Chemomunotherapy with fludarabine, cyclophosphamide and rituximab (FCO) is the optimal front-line treatment for fit chronic lymphocytic leukemia (CLL) patients. IGHV mutations and FISH lesions are predictive markers of response and progression-free survival after FCR. Minimal residual disease (MRD) is the single best post-treatment predictor of long-term outcome after FCR, independent of biologic prognostic markers.

Aims: To explore whether conventional biologic markers (i.e. IGHV mutations, FISH lesions) and TP53, NOTCH1, BIRC3 and SF3B1 mutations can predict the attainment of a MRD negativity after first-line treatment of CLL patients with FC and ofatumumab (FOC).

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.

Results: Sixty-five responding patients underwent MRD evaluation at month +8; their biologic features are reported in the Table. By flow cytometry, 25/65 (38%) resulted MRD+ in the PB and/or BM, while the remaining 40 (62%) showed no residual CLL cells. The absence of del17p+TP53mut/del11q was associated with the achievement of MRD negativity: 37 MRD- (74%) vs 13 MRD+ among patients with trisomy 12/negative FISH/del13q/TP53WT vs 2 MRD- (14%) vs 12 MRD+ among del17p+/TP53mut/del11q+ (p=0.0001). Interestingly, when patients were stratified into high (n=6), intermediate (n=22), low (n=21) and very-low risk (n=15) groups by integrating FISH and gene mutations (Rossi et al., Leukemia 2010), 18/22 (82%) vs 16/39 (41%) vs 10/21 (48%) vs 5/15 (33%) resulted MRD- (Fisher’s exact test p<0.05).

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et al., 2013), the high and intermediate risk groups (del17p/TP53/BIRC3+) or del11q/NOTCH1/538F+) showed a significantly lower probability of achieving a MRD negativity (36%, 10/28) than the low and very low risk groups (+12/ negative FISH/del13q/WT for 4 genes: 81%, 29/36) (p=0.0003). The 40 flow cytometry MRD- cases were also evaluated by ROC-PCR: 22 (55%) were reclassified as MRD+. By combining the two methods, 47/65 cases (72%) were MRD+ and 18/65 (28%) MRD- at the end of FCO. Mutated (M)-IGHV status was signifi-
cantly associated to a molecular MRD- (12 MRD-15 MRD+, 44%) compared to unmutated (UM)-IGHV cases (5 MRD-/32 MRD+, 13%) (p=0.0092). Moreover, when M-IGHV status is reinforced by the absence of del17p/ TP53/ndel11q, the association with a deeper MRD negativity got stronger (12 MRD-15 MRD+, 44% vs 5 MRD-/32 MRD+, 13%; p=0.0038). A multivariable model including FISH lesions, gene and IGHV mutations supports the inde-
pendent role of FISH and IGHV profile in predicting MRD negativity by flow and ROC-PCR, respectively.

### Summary/Conclusions:

In CLL patients treated with the FCO combination (L3), the Gata regulation of MRD negativity by flow cytometry (82%) can be predicted by the FISH profile: 74% in patients without del17p/ndel11q vs 14% in del17p+/del11q+ cases. A deeper MRD negativity by ROC-PCR (28%) can be anticipated by the IGHV status (44% M vs 13% UM) or by combining IGHV and FISH. A longer follow-up will determine whether these parameters can identify patients who maintain over time a good quality of response.

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**E1001**

**ISOCROMOSOME 17q, UNBALANCED TRANSLocations AND 8q GAIN REPRESENT adverse prognostic factors in chronic lymphocytic leukemia (CLL)**

**Summary/Conclusions:** The type of chromosomal abnormality leading to 17p- is a prognostic factor, especially del17p+. However, the remaining allele being generally mutated. The presence of additional gene abnormalities accompanying the 17p- unbalanced translocations were found in 121/195 (63%) of patients. Combining FISH and K, del13q was detected in 71/118 (60%) of cases, del8p in 40/189 (21%), tril2 in 30/195 (15%), gain8q in 13/105 (12%), and del11q in 20/161 (12%). By univariate analysis, the parameters which were associated with significantly shorter follow-up were: age ≥65 years (p=0.0092); and gain8q-17p+ (p=0.0003). The 40 flow cytometry MRD- cases were also evaluated by ROC-PCR: 22 (55%) were reclassified as MRD+. By combining the two methods, 47/65 cases (72%) were MRD+ and 18/65 (28%) MRD- at the end of FCO. Mutated (M)-IGHV status was significantly associated to a molecular MRD- (12 MRD-15 MRD+, 44%) compared to unmutated (UM)-IGHV cases (5 MRD-/32 MRD+, 13%) (p=0.0092). Moreover, when M-IGHV status is reinforced by the absence of del17p/ TP53/ndel11q, the association with a deeper MRD negativity got stronger (12 MRD-15 MRD+, 44% vs 5 MRD-/32 MRD+, 13%; p=0.0038). A multivariable model including FISH lesions, gene and IGHV mutations supports the inde-
pendent role of FISH and IGHV profile in predicting MRD negativity by flow and ROC-PCR, respectively.

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**E1002**

**THE MICROENVIRONMENT REGULATES THE EXPRESSION OF miR-21 AND TUMOR SUPPRESSOR GENES PTEN, PIA33 AND PDCD4 THROUGH NOTCH1 AND BIRC3**

**Summary/Conclusions:** The type of chromosomal abnormality leading to 17p- is a prognostic factor, especially del17p+. However, the remaining allele being generally mutated. The presence of additional gene abnormalities accompanying the 17p- unbalanced translocations were found in 121/195 (63%) of patients. Combining FISH and K, del13q was detected in 71/118 (60%) of cases, del8p in 40/189 (21%), tril2 in 30/195 (15%), gain8q in 13/105 (12%), and del11q in 20/161 (12%). By univariate analysis, the parameters which were associated with significantly shorter follow-up were: age ≥65 years (p=0.0092); and gain8q-17p+ (p=0.0003). The 40 flow cytometry MRD- cases were also evaluated by ROC-PCR: 22 (55%) were reclassified as MRD+. By combining the two methods, 47/65 cases (72%) were MRD+ and 18/65 (28%) MRD- at the end of FCO. Mutated (M)-IGHV status was significantly associated to a molecular MRD- (12 MRD-15 MRD+, 44%) compared to unmutated (UM)-IGHV cases (5 MRD-/32 MRD+, 13%) (p=0.0092). Moreover, when M-IGHV status is reinforced by the absence of del17p/ TP53/ndel11q, the association with a deeper MRD negativity got stronger (12 MRD-15 MRD+, 44% vs 5 MRD-/32 MRD+, 13%; p=0.0038). A multivariable model including FISH lesions, gene and IGHV mutations supports the inde-
pendent role of FISH and IGHV profile in predicting MRD negativity by flow and ROC-PCR, respectively.

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**Background:** Microenvironment found in bone marrow and lymph nodes supports survival, proliferation and drug resistance in chronic lymphocytic leukemia (CLL). Indeed, CLL cells are highly dependent on interactions with the microen-
vironment. The BCR is one of the key players involved in the crosstalk between CLL cells and the microenvironment. Furthermore, it has a critical role in patho-
genesis and prognosis of CLL. Accordingly, different factors related to increased BCR signaling are adverse prognostic factors in CLL, such as IGHV genes, high expression of ZAP-70 and increased serum levels of CCL3. Expression of ZAP-70 in CLL cells has been related to enhanced response to BCR stimu-
lation, as well as to increased response to diverse migrative and survival stimuli from the microenvironment. MiR-21 is an oncogenic microRNA that has been found to be overexpressed in a wide variety of neoplasms where it participates in oncogenic events such as proliferation, resistance to treatment, and meta-
tasis; its overexpression in CLL has been associated to refractoriness to flu-
conazole and to shorter overall survival and higher probability of progression. Aims: In order to further elucidate the molecular mechanisms defining bad prognosis CLL by further elucidation of the role of ZAP-70 in the crosstalk between CLL cells and the microenvironment, we studied the relationship between ZAP-70 protein and miR-21 and how it is influenced by the microen-
vironment. From this, we can hypothesize a model of chromosomal abnormalities that may be involved in the crosstalk between CLL cells and the microenvironment.

**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) and MAPK (PD98059) and STAT3 (USI-124) inhibitors for 1 hour. BCR was stimulated with F(ab)2 anti-IgM. PBMC were co-cultured with bone marrow stromal cells with CD40L and CpG to mimic the microen-
vironment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels of miR-21 were evaluated by primary miR-21, miR-21, PTEN, PDCD4 and PIA33 were measured by QRT-PCR.

**Results:** First, we observed that miR-21 expression was significantly higher in patients with high expression of ZAP-70. Subsequently, using stably transfected Ramos B-cells with ZAP-70 protein we found that pri-miR-21 and mature miR-
-21 were significantly increased upon ZAP-70 stimulation, as well as enhanced by ectopic expression of ZAP-70. We also observed that inhibition of both MAPK and STAT3 pathways impairs the regulation of miR-21 expression after ZAP-70 activation. Moreover, the induction of miR-21 expression after ZAP-70 activation also induced downregulation of the tumor suppressor gene PTEN, as well as increased expression of miR-21. Moreover, we observed that miR-21 was up-regulated in the microenviron-
ture of primary CLL cells induced ZAP-70 and miR-21 expression, as well as downregulation of the putative miR-21 targets. Interestingly, the increase in miR-21 after co-culture was significantly impaired by ibrutinib, indicating that miR-21 expression was modulated by the microenviron-
ment. From this, we can hypothesize a model of chromosomal abnormalities that may be involved in the crosstalk between CLL cells and the microenvironment.

**Summary/Conclusions:** In conclusion, stimuli from the microenvironment are capable of regulating expression of miR-21 and tumor suppressor genes
E1003 IMPACT OF RECURRENT MUTATIONS ON PROGRESSION-FREE SURVIVAL IN CLL PATIENTS TREATED WITH FRONT LINE RITUXIMAB-BASED REGIMENS

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Background: Regimens consisting of rituximab and DNA-damaging drugs represent an important therapeutic option for patients with chronic lymphocytic leukemia (CLL). Up-to-date studies including clinical trials agreed upon the advantage of TKP53-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 F-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 unmethylated IGHV. The next generation sequencing using MiSeq (Illumina) was done in 53 CLL patients, with 41 relapsed samples using three different panel sets: ATM (exons 2-63; 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 (n=23) harbored all the mutations (wt). The most frequently affected gene was ATM (15 cases; only mutations with predicted functional impact considered), followed by SF3B1 (10 cases; hot-spot mutations), NOTCH1 (7 cases; all deletion c.7541-7542) and BIRC3 (5 cases; frame-shift mutations). We did not observe significant differences in PFS among the employed regimens: the median PFS was 30.5 months and the PFS rates at 5 years were 42.2% (SD 18.7; P=0.96) in the ASCT arm versus 44.0% (SD 18.5; P=0.96) in the chemotherapy arm. Our pilot analysis with limited number of samples (n=13) demonstrated that patients with mutated TP53 have significantly shorter median survival (15.5 m; P=0.041). Analysis of clonal evolution in patients who relapsed (41 paired samples) showed that: (i) only one sample showed no significant change in the number of mutated clones (n=13); (ii) in a more detailed analysis, we observed that the frequency of mutations in ATM gene was higher in late relapse (3% vs 18%) and in patients who relapsed at different timepoints (9% vs 16%); (iii) overall, we observed that the number of mutations in ATM gene as well as their frequency in early relapse (20% vs 42%) and late relapse (5% vs 13%) was significantly higher in patients who relapsed (P<0.05).

Summary/Conclusions: Our pilot analysis with limited number of samples does not indicate an adverse impact of mutations in ATM gene on PFS. In the ongoing analysis, we aim to evaluate impact of mutations in ATM, SF3B1, NOTCH1 and BIRC3 genes on PFS in a larger number of patients treated with front line rituximab-based regimens. We also plan to analyze clonal evolution of mutations in relapse, as well as their impact on survival.

E1004 BCR SIGNALLING PROFOUND CHRONIC LYMPHOCYTIC LEUKAEMIA B CELLS ARE PRONE TO RITUIMAX 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 leukemia (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 CD56. In our study, RTX-treated and untreated CLL cells were co-cultured in the presence of fibroblasts. We showed that RTX significantly increased CXCR4 expression (~2-fold, P<0.005) which was coupled with higher responsiveness of the CXCR4+CD56+CD20- subpopulation, since CD20 was proposed to play a role in BCR signalling. We observed that CXCR4+CD56+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, CXCR4+CD56+CLL cells also have higher levels of CD19 (1.8-fold, P=0.001), which is an important component of BCR complex that augments signal transduction. Furthermore, we demonstrated that CXCR4+CD56+CLL cells have higher phosphorylation of several proteins involved in PI3K/BCR/NFkB signalling pathway (P<0.05) compared to CXCR4+CD56+CLL cells obtained from the same patient. This led us to hypothesize that the regulation of BCR signalling is likely of physiological importance for PI3K/BCR signalling. Indeed, we observed significant reduction in phosphorylation of tyrosine-protein kinases associated with PI3K/BCR signalling after silencing of CD20 by siRNA in B cells.

Summary/Conclusions: We showed that CXCR4+CD56+CLL subpopulations in 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.

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E1005 REGULATION OF BCR SIGNALLING IN CHRONIC LYMPHOCYTIC LEUKAEMIA: ROLE OF E3 UBQUITIN LIGASE C-CBL

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Background: in normal B cells, the E3 ubiquitin ligase Cbl (c-Casitas B-lineage lymphoma) is involved in the ubiquitin-dependent Lyn degradation and in the down-regulation of BCR signalling. c-Cbl is activated by phosphorylation that releases c-Cbl from its autoinhibited structure by triggering a conformational change that leads to an enhanced transfer of ubiquitin from the E2 enzyme to the substrate proteins. Mutations in this RING/Linker region result in the loss of ubiquitin E3 ligase activity thus prohibiting lysosomal or ubiquitin/proteasome-mediated degradation of tyrosine kinases and thereby unleashing tyrosine kinase signalling. We reported that in Chronic Lymphocytic Leukemia (CLL) Lyn is over-expressed and is in an active conformation as integral component of an aberrant cytosolic multicomponent complex, associated with several proteins, (CXCR4, SDF1, HS1 and Calpain I among others) that can contribute to cytosolic Lyn, thus stabilizing the aberrant complex and converting individual transient interactions into stable ones. Aims: The accumulation of clonal B lymphocytes in CLL is mostly due to apoptosis resistance but also to proliferative activity. Abnormalities of molecules involved in signal transduction associated with lymphoid cells and a strongly reduced and a critical role has already been ascribed to B-cell receptor (BCR)-Lyn axis. Here, we investigated the expression and the role of c-Cbl in CLL B cells since in normal B cells it is involved in the ubiquitin-dependent Lyn degradation and in the down-regulation of BCR signalling.

Methods: Peripheral blood samples were collected from 30 CLL patients and 15 controls. Untouched peripheral blood B cells were purified using the RosetteSep isolation kit for human B cells. We characterized c-Cbl total protein level and c-Cbl(Y700) by Western blotting. To evaluate the interaction between c-Cbl and Lyn in CLL...
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 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 mis-regulation 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 and an active pool in the cytosol which is dependent on Lyn activation, after 24 and 48 hours with/without a layer of Mesenchymal Stromal Cells (MSCs). Caspase dependence was demonstrated using the pan-caspase inhibitor z-VADfmk.

**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[f4-(3-hexyloxy)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.

**Results:** We performed in vitro phosphatase activity assays on the cytosolic pool of SHP-1 in the presence of increasing concentrations of nintedanib, a receptor tyrosine kinase inhibitor recently shown to trigger SHP-1 activity. Nintedanib treatment could activate the phosphatase (at Ser951), and inhibited, form of SHP-1 and to induce apoptosis, depending on the caspase activation, after 24h and 48h at marked level. Interestingly, we recently demonstrated that Ser91 phosphorylation of SHP-1 could be dephosphorylated by MP07-66. In this scenario, the restoration of PP2A activity by a fingolimod analogue 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 stimulation of each one or 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 tumour microenvironment.

**Summary/Conclusions:** In conclusion, our findings indicate that phosphatase activators may represent a new weapon against this form of leukaemia. 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 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 over-expressed 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 in 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. Assessments tested: CXCR4 binding-affinity (flow cytometric competition assays), cell-binding characteristics (immunocytotoxic fluorescence) and blockade of CXCL12-induced signalling (immunoblotting). 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(cyc)lam drug Plerixafor). The studies confirmed BAT1 had high affinity for CXCR4 receptors expressed on primary CLL cells. Immunocytotoxic fluorescence comparison with its native ligand confirmed binding of BAT1 to the CLL cell surface, while immunoblotting demonstrated binding of CXCL12-induced downstream 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(cyc)lam drugs. This work has been extended to attach BAT1 to liposomes, with present work optimising liposome characteristics for binding and uptake by CLL and the delivery of cytotoxic payload.

**Figure 1.**

**Summary/Conclusions:** A novel bis(cyc)lam CXCR4 antagonist and targeting agent (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
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. TPO-R (CD110) expression on CD4+ T-cells was estimated by flow cytometry. CD4+ T-cells were incubated with low-dose IL2 and TPO for 5 days. Percentage of cells retained in G0 (non-proliferating pool) was determined. CLL B-cells were co-cultured with a TLR9 agonist (ODN) and TPO expression was assessed by Q-PCR.

Results: CD4+ T-cells of CLL patients expressed significantly higher levels of TPO-R (CD110) compared to T-cells of healthy donors, with a mean fluorescent intensity of 764±148 and 498±206, respectively (p<0.05; n=6). Stimulation of patient T-cells with TPO induced an increase in the number of cells retaining in G0 (from 75±5.4% to 86±5.6%; p<0.05; n=8), whereas proliferation of healthy donor T-cells remained unaffected by TPO (11.5%±5.7% and 11.4%±5.7% of G0; 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<0.05; 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 cells could be the TPO source in this disease, TPO mRNA expression in the malignant cells was assessed, demonstrating a baseline ct value of 72±1296, 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 B-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 cirtumzumab can enhance treatment efficacy in CLL. Nevertheless, the variability in ROR1 expression during disease progression, therapy administration and relapse remains unknown.

Aims: In our study we aimed to i) detect ROR1 in CLL cells during different stages of the disease using flow cytometry and qRT-PCR with focus on patients undergoing therapy; ii) 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 ibrutinib, 13 patients treated with idelalisib. To monitor 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-color flow cytometry (modified MRD protocol: CD45/CD3/CD19/CD5/CD8/CD79b/CD22/ROR1) in all patients. To quantify ROR1 mRNA expression changes within individual patients we performed qRT-PCR in separated CLL cells (>95% CD19+CD5+). CLL cells from samples in remission were separated immunomagnetically (Whole Blood Anti-ROR1 MicroBead, Miltenyi Biotec).

Results: Using multicolour flow cytometry we confirmed ROR1 antigen/protein expression in CLL cells in all studied samples. ROR1 antigen was detectable on residual CLL cells during disease remission with the ability to distinguish malignant population from healthy B-cells. Using qRT-PCR we detected significantly higher levels of ROR1 mRNA in samples of treated patients (p<0.01). This observation was supported by analysis of ROR1 mRNA expression in five patients tested consecutively in several time-points. We detected ROR1 mRNA expression increase in disease progression before therapy and further increase during therapy administration. In case of remission induction we observed decrease of ROR1 mRNA level. In patients treated with ibrutinib or idelalisib we observed steep increase of ROR1 expression compared to patients treated with other regimens.

Summary/Conclusions: ROR1 protein 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.

mediated TFS of 4 years (log rank test: p=0.0003). Thus, patients with normal SPE and IGHV mutated status constitute a group with excellent prognosis.

Summary/Conclusions: In conclusion, normal SPE was associated with good outcome with decreased accumulation of side genetic events (in particular, SF3B1 mutations). This analysis shows a bias in IGHV repertoire according to SPE status. These results also clearly suggest that patients with a normal SPE and mutated IGHV have an extremely quiet CLL natural history. This could be either due to the weaker activity of the disease and/or to the absence of adverse consequences of a concomitant paraprotein.

E101

HS70 EXPRESSION IS MODULATED BY ITS MASTER REGULATOR HSF1 VIA MAPKS AND PI3K/AKT/MTOR PATHWAYS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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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. We recently found that the Heat Shock Protein of 70kDa (HS70), 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 main responsible for the transcription of HS70, is itself overexpressed in CLL and strictly correlated to HS70. In response to stress, HSF1 becomes phosphorylated, forms homotrimers, binds DNA and activates heat shock gene transcription. HSF1 is regulated by a finely tuned balance of activatory/inhibitory phosphorylations mediated by kinases belonging to pathways triggered by RAS (i.e. PI3K/AKT/mTOR and RAF/MEK/ERK).

Aims: Since HS70 is overexpressed in CLL neoplastic B cells and most of ‘HSF1-phosphorylating actors’ belong to signalling pathways taking part from RAS, being the PI3K/AKT/mTOR and the RAF/MEK/ERK pathways, we are herein aimed at gaining information and dissecting this network in CLL B cells.

Methods: In a Reverse Phase Protein Array (RPPA) study, previously performed from 57 CLL patients and 11 healthy subjects, we assessed the activation/expression of key signalling proteins. Herein, we focused on HS70, AKT-Ser473, mTOR-Ser244, GSK3α/b-Ser21/9, CDK2, CREB-Ser133, Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. RPPA and demonstrated that the examined proteins behave in a different way as cut-off the value of the median of HS70 expression levels calculated by RPPA and demonstrated that the examined proteins behave in a different way between patients expressing high or low levels of HS70. HS70-high patients present high Akt-Ser473, an inhibitor of GSK3a/b that, in the inhibited form, prevents HSF1 inhibition. By contrast, HS70-low patients have high MEK1/2-Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. Intriguingly, p38MAPK-Thr180/Tyr182, SAPK-JNK-Thr183/Tyr185 and PDK1-Ser241. Cluster and separated analyses have been performed.

Results: We divided our patients in HS70-high and HS70-low considering as cut-off the value of the median of HS70 expression levels calculated by RPPA and demonstrated that the examined proteins behave in a different way between patients expressing high or low levels of HS70. HS70-high patients present high Akt-Ser473, a regulator of GSK3a/b that, in the inhibited form, prevents HSF1 inhibition. By contrast, HS70-low patients have high MEK1/2-Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. Intriguingly, p38MAPK-Thr180/Tyr182, SAPK-JNK-Thr183/Tyr185 and PDK1-Ser241. Cluster and separated analyses have been performed.

Summary/Conclusions: These data would suggest that, in CLL, HS70 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 HS70 while an activation of the RAF/MEK/ERK signalling rather results in HS70 down regulation. The dissection of signalling pathways connected to HS70-HSF1 axis in CLL will contribute to define the biology and understand the pathogenesis of this disease.

E102

THE INTERPLAY BETWEEN TH17 AND TREGS: A NEW IMMUNOSUPPRESSIVE INSIGHT IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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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 (o/n), followed by a 5 h stimulation with PMA, ionomycin and Monensin (PIM), or with anti-CD4 FITC, anti-CD25 APC-Cy7, anti-FoxP3 APC and anti-Tbet PE or anti-ROTYt 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+ CD25+IL-10+FoxP3+ cells (Tregs) in CLL samples, with a statistically significant increase in Th17, Th1, Th2, and Th17/Treg subsets in CLL patients, probably trying to overcome the deficit of effector T cells. In order to study regulatory T cells, we evaluated the ability of CD4+ T cells to secrete IL-10 or both. We observed a statistically significant increase in the frequency of CD4+ IL-10-producing cells in patients than HV, whereas the percentage of IL-17A+/IL-10- cells remained unchanged.

Summary/Conclusions: Our results highlighted the importance of the Th1/Th2/Th17/Treg network in the immune modulation of CLL. The observed modifications in CD4+ T cells may represent an adaptive mechanism to avoid the presence of TH17 cells and to maintain a Th2 response in order to downregulate the immune system.

E103

LOW EXPRESSION OF CD25 IN CHRONIC LYMPHOCYTIC LEUKEMIA NOTCH1-MUTATED CASES INDEPENDENT OF CDK4/6 MISREGULATION

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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 mutant status detected by deep sequencing in a cohort of 138 patients, and to correlate it with the immunophenotypic profile and CDK4 and CDK6 expression.

Methods: We performed targeted NGS sequencing of blood samples, collected at diagnosis, from 138 CLL patients. We designed a TruSeq Custom Amplicon sample preparation kit, with a list of~51 target regions of the genome that include~1100 genes. We performed un-parametric Wilcoxon signed-rank test.

Results: The aim of this study is to clarify the interplay between TH17 and Tregs in the pathogenesis of CLL.
containing 13 genes and evaluating 28,099 bases. Paired-end sequencing was performed with MiSeq v2 chemistry, and a mean depth of 96x across the targeted region was obtained. Every patient underwent, at baseline, a flow cytometry characterization with a panel including (slg)k, (slg)λ, CD19, CD11b, CD81, CD10, CD79b, CD29, CD38, FMC7, CD22, CD45, CD103, CD11c, CD25, ZAP70, CD11a, and CD24. CDK4 and CD6K expression levels were quantified by RT-qPCR.

**Results:** With a median age of 66 y.o. (range, 31-89) and a slight male predominance, the median follow up time of our cohort was 43 months (24-104). We found that 38/138 (28%) patients harbored at least one mutation, with NOTCH1 (n=16, 12%), ATM (n=12, 9%), TP53 (n=9, 7%), and SF3B1 (n=8, 6%) among the most commonly mutated genes. Those patients with a NOTCH1 mutation showed a lower CD25 expression (24 mean fluorescence intensity units (MFIu)) than those without a mutation (43 MFIu), *p*=0.03. We could not validate the recently reported association between the presence of NOTCH1 mutations and a low expression of CD25. In our cohort, the MFI in NOTCH1 mutated and non-mutated patients was 163 and 146 units, respectively (*p*=0.05). We measured CDK4 and CD6K 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). We noted 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 to be present 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 CD4K and CD6K regulators.

**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 to be present 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 CD4K and CD6K regulators.

**E1014**

**GENE MUTATIONS ANALYZED BY NEXT-GENERATION SEQUENCING ALLOW US TO DEFINE THE PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH EARLY-STAGE DISEASE AND 13Q DELETION**

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**Background:** Next-Generation sequencing (NGS) studies have revealed a number of recurrently mutated genes in chronic lymphocytic leukemia (CLL). It is reasonable to argue that evaluating the newly gene mutations as prognostic markers would help to improve prognostic counseling of CLL patients. Interestingly, gene mutations could help us to refine the prognosis in the group of CLL patients with other prognostic markers associated with good prognosis.

**Aims:** To analyze the presence of mutations of a panel of genes by NGS and its prognostic impact in patients with CLL, focusing in the groups of patients with good prognosis characteristics.

**Methods:** Amplionc-based-NGS was performed using 454 platform in 147 CLL patients to evaluate the mutational status of 6genes (TP53, NOTCH1, SF3B1, XPO1, FBXW7 and MYD88). Samples were obtained at diagnosis or before treatment in all cases. 70.1% were Binet A and 53% had 13q deletion (13q-). A cut-off 2% was applied to define variants. All the mutations were validated.

**Results:** 1. NGS analysis showed that 37.4% of CLL patients (55/147) showed mutations in any of the analyzed genes. The frequency of mutations was 16.3% for NOTCH1, 10.2% for SF3B1, 6.8% for TP53, 4.8% for XPO1, 3.4% for FBXW7, and 1.4% for MYD88. The presence of mutations in any of these genes except to MYD88 (mutated CLL) was significantly associated with clinical progression (60.0% for mutated CLL vs. 38.2% for unmutated CLL, *p*=0.05). Interestingly, mutated CLL patients showed a shorter time to first treatment (TFT) than unmutated CLL patients (30 months vs. 88 months; *p*=0.006). By contrast, MYD88 mutations were detected in CLL with mutated IGHV and 13q-. Of note, 23.6% of the mutations had a mutational load of ≤15% and thus would not have been detected by capillary Sanger sequencing. CLls with mutations in MYD88 had an inferior TFT than those without mutations (18 vs 88 months; *p*=0.018), and similar to CLL patients with mutations >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 Binet A and 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 unmutated 13q- CLL patients (22 vs 88 months, *p*=0.002).

**Summary/Conclusions:** 1) CLL patients with mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 show a worse prognosis than CLL patients without mutations. 2) Gene mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 in a low percentage of the cells are associated with a shorter TFT. 3) Among CLL patients with good prognostic characteristics (Binet A and 13q-), gene mutations help us to define the prognosis of the patients.

**E1015**

**ALTERED COMPLEX C5 IS ASSOCIATED WITH COMPROMISED COMPLEMENT ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background:** The therapeutic monoclonal antibodies used for the treatment of Chronic lymphocytic leukemia (CLL) mediate anti-tumor effects through several mechanisms: complement cell-mediated cytotoxicity (ADCC), and phagocytosis. CDC efficacy thus depends on the expression level of the target B-cell antigen, the integrity of apoptotic cascades within tumor cells, the functional capacity of effector cells, and the availability and activity of the complement (C) system. Published data indicate deficiency of one C protein or more in most CLL patients, as well as additional factors that may affect C activity. The role of structural abnormalities of C complexes in affecting C function has not been investigated.

**Aims:** To study the structural integrity of circulating C complexes, focusing mainly on C5, and to establish its importance for C activity in CLL. **Methods:** Blood samples were collected from 35 (20) Binet A and 10 healthy controls (HC). Biochemical and haematological parameters, and CLL staging were recorded. The isoforms of two C components, C3 and C5 were studied by Western blot analysis. The activity of the C system before and after in-vitro activation via the classical or alternative pathways was followed by the levels of C5b-9, the terminal product of C activation. C activation was also studied in vitro on normal and CLL sera. The differences in C5 pattern were noted in some of the CLL patients. Specifically, the C5 complex that exists as a single protein band in all HC and in 56% of CLL patients appeared in 44% of the patients as a clear double-band. No clear differences were observed in C3 pattern in the patients. Higher basal levels of C5b-9 were found in CLL patients with abnormal pattern of C5 (394±6758 SFM g/mL) compared with both HC (50±158 SFM g/mL) and CLL patients presenting normal C5 pattern (236±655 SFM g/mL). In-vitro C activation via the classical pathway was inversely correlated with basal activity, and was significantly lower (*p*<0.03) in the CLL patients with altered C5 compared to HC and CLL patients with normal C5. In-vitro activation via the alternative pathway was similar in all subjects’ groups. C activity in C5-deficient serum supplemented with 33% sera from patients with abnormal C5 was significantly lower compared to the activity observed after supplementation with serum from HC or from patients with normal C5. Activity after supplementation with normal C5 (commercial) was significantly lower in sera from CLL patients compared to sera from the other subjects’ groups.

**Summary/Conclusions:** The data indicate a possible link between the activation potential of the C system in CLL patients and alterations in the complex structure of C5. The differences in C activation via the classical and alternative pathways may indicate disturbance in the classical pathway in patients with abnormal C5. The exact mechanisms by which abnormal C5 distracts the C activity need further clarification. Yet, the appearance of abnormal C5 in CLL patients with disturbed C activity bears the potential to develop a marker which will assist in identifying patients who are likely to be less responsive to future immunotherapy treatment due to compromised CDC. Development of such a marker may assist clinicians in refining and personalizing the immunotherapeutic approach, improving CDC and consequently the therapy results.
Chronic lymphocytic leukemia and related disorders - Clinical

E1016
ASSOCIATION OF CGP-STIMULATED KARYOTYPE WITH TIME-TO-FIRST TREATMENT FOR CLL
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Background: Prognostic factors correlate with clinical outcomes, independent of treatment. B cell receptor (BCR) signaling pathway inhibitors can nullify the prognostic impact of some markers, such as IGHV mutation status. CpG-stimulated metaphase karyotype can identify clonal cytogenetic abnormalities in CLL that may not be seen with standard non-stimulated karyotype or by FISH. Complex cytogenetics, defined as 3 or more chromosome abnormalities in 2 or more metaphases was the highest-risk feature for shorter progression-free and overall survival in patients receiving ibrutinib for relapsed/refractory CLL. Complex karyotype is not uncommon among relapsed/refractory CLL cases, particularly those who previously received genotoxic chemotherapy.

Table 1. Continuous and Categorical Patients Characteristics.

<table>
<thead>
<tr>
<th>Continuous Characteristic</th>
<th>n</th>
<th>Number (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range) (yr)</td>
<td>540</td>
<td>62 (19-91)</td>
</tr>
<tr>
<td>IGHV mut status</td>
<td>498</td>
<td>20.5 (2.5-59.9)</td>
</tr>
<tr>
<td>β2M</td>
<td>497</td>
<td>8.2 (1.4-10.9)</td>
</tr>
<tr>
<td>IGHV mut status (tall)</td>
<td>493</td>
<td>39.3 (39.8)</td>
</tr>
<tr>
<td>Category</td>
<td>488</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Rai Stage</td>
<td>519</td>
<td>50 (98)</td>
</tr>
<tr>
<td>≤10</td>
<td>75</td>
<td>16 (40)</td>
</tr>
<tr>
<td>≥11</td>
<td>443</td>
<td>84 (65)</td>
</tr>
<tr>
<td>Unmutated</td>
<td>443</td>
<td>81 (85)</td>
</tr>
<tr>
<td>Monoclonal</td>
<td>443</td>
<td>81 (85)</td>
</tr>
<tr>
<td>FISH</td>
<td>443</td>
<td>81 (85)</td>
</tr>
<tr>
<td>Del17p</td>
<td>33</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Del17q</td>
<td>33</td>
<td>7 (7)</td>
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<tr>
<td>Del11q</td>
<td>78</td>
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</tr>
<tr>
<td>Del11p</td>
<td>104</td>
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<tr>
<td>Complex</td>
<td>35</td>
<td>7 (7)</td>
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<tr>
<td>Complex2</td>
<td>16</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Other</td>
<td>83</td>
<td>17 (33)</td>
</tr>
<tr>
<td>Diploid</td>
<td>347</td>
<td>69 (69)</td>
</tr>
<tr>
<td>Positive</td>
<td>124</td>
<td>40 (77)</td>
</tr>
<tr>
<td>Negative</td>
<td>306</td>
<td>60 (60)</td>
</tr>
<tr>
<td>WBC ≤4.0</td>
<td>341</td>
<td>65 (65)</td>
</tr>
<tr>
<td>WBC &gt;4.0</td>
<td>147</td>
<td>27 (27)</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-naive 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-685 (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 de17p, 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%). Study 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. 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) was proposed for evaluating. Recently, Beijing and Barcelona-Breno CLL 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 hybridization (FISH)-detected cytogenetic abnormalities were available for 1299 cases. Del17p was used as the sole marker of TP53 status. The CLL-IPI and the Barcelona-Brno prognostic model were calculated using the methods proposed. The accuracy of the prognostic models was assessed by the Harrell C index (an index of discrimination), the explained variation in mortality (an index combining discrimination and calibration), 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%). Among the 1299 patients, 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 1). In comparison to the Barcelona-Brno prognostic model, 58.1% of the 1299 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). Accordingly, the explained variation in mortality provided by the CLL-IPI was 42% (P<0.001), a figure higher than that due to the Barcelona-Brno prognostic model.
(21%, P<0.001), indicating that the CLL-IPI had a higher prognostic accuracy for mortality compared to that of the biomarkers-only prognostic model. Then, we also compared the ability of the two scores to predict TTFT in newly diagnosed patients. The Harrell C-index of the Barceloña-Brno prognostic model was 0.70 (P<0.001), lower than that of the CLL-IPI score (0.73, P<0.001). The AIC showed the superiority of the CLL-IPI compared to the Barceloña-Brno prognostic model in predicting TTFT (CLL-IPI, AIC=6010.929 versus biomarkers-only prognostic model, AIC=6010.929). Accordingly, the explained variation provided by the CLL-IPI was 33% (P<0.001), a figure higher than that achieved by the Barceloña-Brno prognostic model (28%, P<0.001), indicating that the CLL-IPI had a higher prognostic accuracy for predicting TTFT as compared to that of the Barceloña-Brno prognostic model.

Summary/Conclusions: Our results confirm the validity of both scores (CLL-IPI and biomarkers-only prognostic model) to predict survival and TTFT among patients with previously untreated CLL. Moreover, we have demonstrated that the CLL-IPI which combines clinical and serological data with biological parameters has a higher accuracy for predicting prognosis and TTFT of CLL patients than the Barceloña-Brno biomarkers-only prognostic model.

E1018
PRELIMINARY RESULTS OF S55746/BCL2L201 (A NEW BCL2 INHIBITOR) IN RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS AND EFFECT OF CALIBRATED MODERATE MEAL ON THE PHARMACOKINETICS

Methods: S55746/BCL2L201 as single agent is being investigated in a phase I (EUDRACT NCT02920697), open-label, multiple-dose, international dose escalation trial. S55746/BCL2L201 was initially administered in fasting condition, once daily (in 21-day cycle) until progressive disease (PD) or unacceptable toxicity. A tumor lysis syndrome (TLS) prevention protocol was implemented. After giving informed consent pts could receive 50 to 2000mg according to a modified version of the Continual Reassessment Method for dose allocation process. In the food effect part of the study, 7 non-Hodgkin’s lymphoma patients received a film coated 200mg tablet under fasting condition (i.e. after 10-hour fasting period) and after a calibrated moderate meal the day after.

Results: As of February 2017, 12 CLL pts have been treated (median age 67 years [range 52-82]). On these 12 pts, 5 presented a bulky disease, 1 a 17p deletion, 4 a 11q deletion, and 1 a p53 mutation. CLL pts were dosed up to 700mg, with a median duration on treatment of 79 days. Median number of prior regimens in CLL pts was 4 (range 2-5). Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. The most frequent (≥2 pts) grade 3/4 adverse events (AEs) were neutropenia (n=2) and thrombocytopenia (n=2). AEs possibly related to the study drug were reported in 4 pts: neutropenia (n=2), neutrophil count decrease (n=1), fatigue (n=1), dyspnea (n=1), gingival bleeding (n=1) and left ventricular ejection fraction (LVEF) decrease (n=1). No clinical or laboratory TLS were reported. One patient in the 700mg cohort experienced a DLT (asymptomatic LVEF decrease grade 2 recovered within 2 weeks). At 700mg, a decrease in lymphocytes count from baseline (≥50%) was observed in 3 out of 4 patients associated with a decrease in the sum of the product of the diameters of lymph nodes (from 23% to 40%). This decrease in lymphocytes count, started from cycle 1, and may be correlated with an induction of apoptosis in CLL cells (4 hours post first dose), detected by flow cytometry in CD19+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-comparative pharmacokinetic analysis of the food effect cohort (5 assessable pts) demonstrated that S55746/BCL2L201 PK is modified by the ingestion of a moderate meal (400-500 kcal with fat contributing to 150 kcal). The median Tmax was delayed from 1.5h to 4h when administered with food. Compared with fasting condition, Cmax and AUC increased by approximately 6-fold following a moderate meal. Based on these results, a protocol amendment to the clinical trial has been implemented in order to further investigate the administration with food in a new dose escalation.

Summary/Conclusions: S55746/BCL2L201 monotherapy showed first signs of activity across the tested dose levels with an acceptable safety profile so far. Based on PK food interaction results, dose escalation in the fed state has started.

E1019
INCREASED VIRUS-SPECIFIC IMMUNE RESPONSES PARALLELED BY A PNEUMOCOCCUS-SPECIFIC IMMUNODEFICIENCY STATE AND HYPOGAMMAGLOBULINEMIA: ALREADY EMERGE IN HIGH-COUNT MONOCL. B LYMPHOCYTOSIS PRIOR TO CLL

Methods: A total of 249 subjects (119 males/130 females; aged 68±11y) including 91 healthy donors, 71 CLL-like MBL, 29 CLL-like MBL and 58 CLL 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-antibodies and specific immunoglobulins against CMV (cytomegalovirus), EBV (Epstein Barr Virus), influenza virus and S.pneumoniae were performed by nephelometry and commercial ELISA kits, respectively. Individuals who had received vaccination against Influenza and/or Pneumococcus were excluded from the analysis of the immunoglobulin-specific titers against the corresponding pathogen, respectively. Plasma CMV and EBV DNA load were determined by real-time qPCR using commercially available kits.

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 titer, 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 (<0.05) for IgG and to a lesser extend also for IgM, when the ratios between the virus-specific IgG/total IgG titer of the same isotype were calculated, except for Influenza-specific IgG, that showed the same trend but without statistical significance. Replicating CMV DNA load, only 3/177 individuals, 1 MBL and 2 CLL, were found to be positive (below the limit of quantitation), while EBV DNA load was detected in plasma from 7/191 (all being Binet A CLL) at median levels of 3.6 copies/μl. In contrast to the virus-specific IgG, IgG plasma levels against S. pneumoniae progressively diminished through progression of the disease, in contrast to the overall lower gammaglobulin levels.

**Summary/Conclusions:** Both MBL and CLL patients present relatively high levels of specific Ig against human host viruses in parallel to progressively lower levels of anti-S. pneumoniae antibodies, which might reflect (asymptomatic) chronic reactivation of humoral immune responses against host viruses and thereby negatively decreased protection against other microorganisms, denoting a severe pathogen-specific humoral immunodeficiency state not reflected by the overall plasma immunoglobulin levels. Alternatively, these results might point out a potential role of ubiquitous viruses in the pathogenesis of the disease. Further analyses are necessary to establish the potential relevance of such asymptomatic humoral immune responses against host viruses in the expansion of the tumor B-cell clone and progression from MBL to CLL.

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**E1020**

**AN EXTENSIVE MOLECULAR CYTOGENETIC CHARACTERIZATION IN HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA IDENTIFIES KARYOTYPIC ABNORMALITIES 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 CpG-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, i.e., at least 3 chromosome aberrations); intermediate group (all other karyotypic abnormalities). A cut-off of 98% homology to the germline sequence to discriminate between IGHV mutated and unmutated cases. Mutational screening was performed with Ion Torrent PGM NGS platform on 20 CLL-related genes by using a 5% cut off.

**Results:** Cytogenetic analysis showed favorable findings in 30 patients, unfavorable in 34 cases and intermediate in 36 cases. A complex karyotype was present in 21 patients. By NGS, 95 somatic mutations were observed in 56/101 (55.4%) cases, 80 nonsense mutations, 5 nonsense mutations and 10 frameshit deletions. 16 cases (15.8%) showed mutations in the TP53 gene, 11 (10.9%) in the NOTCH1 gene, 10 (9.9%) in the SF3B1 gene, 8 (7.9%) in the ATM gene, 5 (4.9%) in the BIRC3 gene, 5 (4.9%) in the PTEN gene, 4 (4.0%) in the MYD88 gene, 4 (4.0%) in the POT1 gene, and 18 (17.8%) cases in the remaining 11 genes. 26/56 (46.4%) mutated patients presented two or more mutations. The presence of mutations was associated with unmutated IGHV status (p=0.040) and the complex karyotype (p=0.047). TP53 disruption correlated with the presence of ≥2 mutations by NGS (p=0.001) and a complex karyotype (p=0.012). By multivariate analysis an advanced Binet stage (p=0.01) and an unfavorable karyotype (p=0.01) predicted for a shorter time to first treatment (TTFT), while TP53 disruption (p=0.019) and unfavorable karyotype (p=0.028) predicted for a worse overall survival (OS). A shorter time to chemorefractoriness (TTCR) was associated with TP53 disruption (p=0.001) and unfavorable karyotype (p=0.025). Patients with both unfavorable karyotype and TP53 disruption presented a dismal outcome (median OS and TTCR of 28.7 and 15.0 months respectively).

**Summary/Conclusions:** A comprehensive analysis of chromosomal aberrations and gene somatic mutations in high-risk CLL showed that the cytogenetic profile was independently associated with a shorter TTFT, OS and TTCR. Since karyotyping using novel mitigogens may contribute to the refinement of prognosis in high-risk CLL patients, the introduction of this technique in future CLL trials seems warranted to identify those patients that could be ideal candidates for consolidation treatment or novel treatment combinations.

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**E1021**

**SHOULD CLL-IPI BE USED TO ASSESS OVERALL SURVIVAL OF EVERY CLL PATIENT? A SYSTEMATIC REVIEW AND META-ANALYSIS**

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**Background:** A weighted grading approach based on five independent prognostic factors (i.e., TP53 status, IGHV mutational status, 8-loc 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 (4563 or 67.7%) came from studies of external validation of CLL-IPI while 17% (1192) and 8.5% (576) had been used to generate (train- and validation phases) and to internally validate the model. Patient distribution in the four risk categories of CLL-IPI was heterogeneous thus reflecting the CLL phase (i.e., at diagnosis, at time of first treatment and at relapse) of patients within different studies. According, 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= 0.00; I2, 87%), 85% for intermediate group (95% CI, 79-82%; Q=49.36; P<0.00; I2, 86%), 60% for high-risk group (95% CI, 57-62%; Q=42.78; P<0.00; I2, 84%) and 32% for very high-risk group (95% CI, 27-38%; Q=18.1; P=0.01; I2, 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 seems warranted to identify those patients that could be ideal candidates for consolidation treatment or novel treatment combinations.
TREATMENT AND 17P DELETION TESTING PATTERNS IN COMMUNITY E1023
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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 questionnaire was developed using the Morsky Medication Adherence Scale® score, which informed nurse coaching frequency. Adherence was delineated by prescription refill compliance. Patient and physician questionnaires were used to gauge satisfaction with the YOU&i™ PSP. Results: As of 20 January 2016, a total of 903 patients with CLL were enrolled in the YOU&i™ PSP. A total of 552 patients were included in the adherence analysis. Of these, 86% opted in to receive the nurse coaching component. At 2 months from treatment initiation, patients who received nurse coaching demonstrated an adherence rate of 92.3%, as compared with 63.5% for patients who did not receive nurse coaching (95% CI, 17.5±41.0; p <0.0001). At 3 months the adherence rates were 89.9% vs 60.8% (95% CI, 17.5±41.4; p <0.0001). By 9 months, adherence rates were 81.7% vs 71.1% (95% CI, -4.4 to 28.4; p=0.141). 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.

Summary/Conclusions: The current analysis provides insight into adherence patterns of patients on long-term ibrutinib treatment. These results are consistent with the literature showing that PSPs like the YOU&i™ PSP can help to improve adherence rates (Schneider SM, et al. J Adv Pract Oncol. 2014;5(3):163-172). The information obtained from long-term adherence data can help to inform future trials examining patterns of adherence with OAMs. Nurse coaching may be helpful in supporting early adherence by addressing side effects that occur more frequently at treatment initiation. Moreover, changes in disease or health status that arise over the first 12 months of therapy may provide information that allows a PSP to adapt to patients' evolving needs over the treatment journey. A better understanding of long-term adherence patterns may allow programs such as the Canadian YOU&i™ PSP to target adherence support more precisely, thereby optimizing patient outcomes.

E1024
SINGLE-AGENT IBRUTINIB VS REAL WORLD TREATMENT FOR PATIENTS WITH TREATMENT-NAÏVE (TN) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): AN ADJUSTED COMPARISON OF RESONATE-2™ WITH THE CLLEAR AND LYON-SUD DATABASES
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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 (cbl) in TN (aged ≥65 years) CLL patients. In the absence of direct comparison of single-agent ibr with other frequently utilized agents in this patient population, this retrospective observational study utilized a propensity score matching analysis based on 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 differences in patient characteristics from RESONATE-2™ vs RW from the CLLEAR (Chronic Lymphocytic Leukemia Registry) and Lyon-Sud databases. Methods: CLLEAR holds medical records for CLL patients from seven academic centers across the Czech Republic. Lyon-Sud database holds medical records for CLL patients from a French 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 compared...
pared between ibru and RW treatment using patient-level data from RES- 
ONATE-2™ (n=136) and pooled patient-level data from the two cohorts. To 
adjust for differences in patient characteristics between the trial population 
and both cohorts, a multivariate Cox proportional hazards model was fitted on 
patient-level data to estimate the hazard ratio (HR) for ibru vs RW treatment, 
with age, sex, disease stage (based on RAI/BINET), and deletion 11q pres-
ence/absence instead as covariates.

Results: Median age at treatment initiation for CLELLR (n=418) and Lyon-Sud 
(n=110) was 73 and 71 years, respectively, vs 73 for ibru patients from RES-
ONATE-2™. The proportion of male patients was 63% in CLELLR and 57% in 
Lyon-Sud vs 85% in RESONATE-2™. The median follow-up was 35.7 months 
(mo) for Lyon-Sud and 16.8 mo in CLELLR vs 29.1 mo for RESONATE-2™. 
Adjusted HR for ibru vs physician choice in CLELLR and Lyon-Sud were 0.23 
(95% CI: 0.14, 0.39) and 0.25 [0.14, 0.43] for PFS, and 0.29 [0.11, 0.79] and 
0.39 [0.18, 0.83] for OS, respectively. Fludarabine/cyclophosphamide/ritux-
imab (FCR; n=117), bendamustine+R (BR; n=91), CH alone (n=43), CH+ 
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 fac-
tors for PFS and OS. The adjusted HRs (pooled estimates) for ibru vs the two 
most commonly used regimens were 0.30 [0.17-0.53] (FCR) and 0.33 [0.16-
0.68] (BR) for PFS, and 0.44 [0.20-0.95] (FCR) and 0.53 [0.13-0.83] (BR) for OS 
(Figure 1). Estimates of HR vs regimens in the cohorts were consistent across 
databases.

Figure 1.

Summary/Conclusions: This adjusted comparison of patient-level data from RESONATE-2™ with RW data from CLELLR and Lyon-Sud demonstrates ibru 
to be more effective compared with RW treatment, with a 4.1-fold improvement 
in PFS and a 3-fold improvement in OS. When comparing ibru with the most 
commonly used RW treatments, statistically significant benefits for ibru were 
consistently observed vs all treatment regimens on PFS and for most compar-
isons on OS. These results further support the existing evidence that ibru sig-
ificantly improves PFS and OS vs common regimens used in TN CLL settings, and has important implications for clinical practice.

E1025

CHARACTERISTICS, TREATMENT, AND OUTCOMES OF ≥80 YEAR OLD PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) ENROLLED TO PROSPECTIVE TRIALS OF THE GERMAN CLL STUDY GROUP (GCLLSG)

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Background: People over 80 years are the fastest growing age group in west-
ern populations. Clinical management of ≥80 year old patients (pts) with CLL 
remains a challenge due to the very limited amount of data currently available 
for this age segment. Two retrospective studies reported observational data on 
characteristics, treatment, and outcomes of ≥80 year old pts not enrolled in a 
clinical trial (Br. Muller et al). Little is known about ≥80 year old pts who were treated for CLL within clinical trials, however.

Aims: To study the characteristics, treatment, and outcomes of pts aged ≥80 
years who received their first therapy within prospective trials of the German 
CLL Study Group (GCLLSG).

Methods: Trial populations of seven clinical trials of the GCLLSG (CLL1, CLL5, 
CLL7, CLL8, CLL9, CLL10, CLL11; total N=3552) were reviewed and screened 
for pts ≥80 years at frontline treatment. Clinical, laboratory, and genetic data of 
identified pts were pooled. Time-to-event analyses were performed by Kaplan-Meier 
methology. Independent prognostic factors for survival were identified by 
multivariate analysis using Cox regression modelling with stepwise selection 
procedures.

Results: Among 3552 reviewed GCLLSG trial participants, 152 were aged ≥ 
80 years at initiation of firstline treatment. A majority of these pts were identified 
from CLL1 (n=132) while the remaining were from CLL1 (n=3), CLL5 (n=1), 
CLL7 (n=3), CLL8 (n=2), CLL9 (n=9), and CLL10 (n=2). Median age was 82 
years (range 80-90). Concomitant diseases were present in 99% of the pts and 
median cumulative illness rating scale (CIRS) score was 8 (0-18). Median 
creatinine clearance was 46 ml/min (range 17-99 ml/min). Identified genomic 
 aberrations were 1q deletion as a sole abnormality in 27%, trisomy 12 in 18%, 
11q deletion in 9%, and 17p deletion in 16% of pts. (IGHV was unmutated in 
69% of the pts. Distribution of CLL-IPPI risk groups was as follows: 6% low, 19% 
intermediate, 61% high, and 14% very high. Most pts had Binet Stage B (36%) 
or C (43%). Chemioimmunotherapy with chlorambucil plus obinutuzumab (CLB-
OB) or chlorambucil plus rituximab (CLB-R) was administered to 61 (40%) and 
56 (37%) pts, respectively. Remaining pts received chlorambucil alone (CLB, 
n=19), fludarabine (F, n=10), fludarabine/cyclophosphamide (FC, n=1), fludara-
bine/cyclophosphamide/rituximab (FCR, n=2), or bendamustine/rituximab (BR, 
n=3). Rates of grade 3 or 4 neutropenia and infections were 35% and 13%, 
respectively. Premature treatment discontinuations occurred in 15% of cases 
and were mostly due to adverse events. The total overall response rate was 
92% with 13% complete remissions. Median observation time for all pts was 
40.7 months. Median progression-free survival (PFS) and treatment-free sur-
vival (TFS) were 17.2 and 32.3 months, respectively. A total of 47 pts (31%) 
received at least one further line of treatment. Median overall survival (OS) 
was 48.3 months, with adverse events (22%) and progressive CLL (16%) being 
the most frequent causes of death. Standardized mortality ratio was calculated 
and showed a 1.99 (CI 1.54-2.53) increased risk of death as compared to an 
age- and sex-matched general population. Independent prognostic factors 
for OS were 17p deletion and elevated serum thymidine kinase.

E1026

THE ROLE OF CD200 IN THE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Clinical, morphologic, immunophenotypic and genetic features 
are the basis for the diagnosis of B-cell malignancies. It is considered that the diag-
nosis of CLL requires the presence in peripheral blood of >5x10⁹/L monocular 
B lymphocytes with a distinctive immunophenotype (i.e. CD19+, CD20+, 
CD23-, FMC7-, CD79a-, CD22+, CD200+, SmIg weak, CD22 weak) each one of them receiving a score of 
1 when the positive cells were >96%. Mean Fluorescence Intensity Ratio 
(FMC7, CD22 and CD79b) are considered score 1 in 

Summary/Conclusions: Findings suggest that anti leukemic therapy (including 
chemoimmunotherapy) is feasible and efficacious in ≥ 80 year old pts with CLL. 
However, such pts are still highly underrepresented in clinical trials and even 
with modern treatment live shorter than age-matched controls of the general 
population. Broader recruitment of these pts to prospective trials and evaluation 
of targeted therapies therefore appears imperative to improve outcome of CLL 
in this age segment.
and type of aberrations detected were compared between techniques. Whole-Genome 2.7M (n=2) or CytoScan HD (n=22) array, results were ana-
mononuclear cells or CD19+ lymphocytes was hybridized to Cytogenetics
4 patients (16%) had received prior treatment. The cohort was enriched in
plexity are lacking.

Background: [1027] KARYOTYPES IN CHRONIC LYMPHOCYTIC LEUKEMIA

E1027

COMPARISON OF CHROMOSOME BANDING ANALYSIS AND GENOMIC
MICROARRAY TECHNIQUES FOR THE DETECTION OF COMPLEX
KARYOTYPES IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Results: Flow cytometry analysis was performed in 99 patients, including 62 cases with a diagnosis of CLL (82.6%) and 37 cases with a “non-CLL” diagnosis (17.4%). Matutes score was 4-5 in all CLL cases and ≤3 in “non-CLL” cases. CD20, CD79 and CD5 were the most consistent markers for CLL (90.3%, 96.8% and 100.0% of sensitivity respectively). Moreover, CD79b and FMC7 had a good discriminant value (80-85% sensitivity). For “non-CLL” cases the most reliable markers were SmIg, FCMI 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 MFIR. In contrast, the accuracy for SmIg significantly increased from 67.7% to 78.5% when using MFIR 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 system 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 1.

Summary/Conclusions: These results confirm CD20 as a valuable marker in the diagnosis of CLL

E1028

ABNORMAL SERUM FREE LIGHT CHAINS RATIO ASSESSMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: A SIMPLE YET POWERFUL TEST CORRELATING WITH CLINICAL OUTCOME AND MINIMAL RESIDUAL DISEASE
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Background: An abnormal serum Free Light Chain (sFLC) ratio has been shown to be significantly associated with poor outcome in chronic lymphocytic leukemia (CLL) [Yegin ZA et al, Eur J Haematol 2010], suggesting that this parameter may discriminate different biological subgroups.

Aims: As the technic is easily implementable in routine lab and cost effective, we evaluated the sFLC levels (kappa + lambda) and kappa/lambda (K/L) ratio in CLL patients in this prospective study. The relationship between abnormal sFLC levels (K+L) and K/L ratio, minimal residual disease (MRD) assessed by flow cytometry (FCM) and disease evolution was evaluated.

Methods: Diagnosis was confirmed by 10-color FCM immunophenotyping of blood lymphocytes on a Navios (Beckman Coulter). Serum FLC kappa and lambda chains were measured by nephelometry using the Freelite™ immunoassay. The normal free kappa chains level was defined as within the range of 3.3-19.4mg/L, and the normal lambda chains level within the range of 5.71-26.30mg/L. A normal sFLC kappa/lambda (K/L) ratio was therefore defined as between 0.26 and 1.65 (a ratio above 1.65 indicating an excess of kappa light chain, and a ratio below 0.26 indicating an excess of lambda light chain). The cumulative level of kappa plus lambda (K+L) was also evaluated. Most patients received combined chemo-immunotherapy or entered clinical trials whenever possible. The ROC methodology was used to establish the best cut-off value of sFLC ratio level to discriminate treated patients from those who remained treatment-free.

Figure 1.

Results: Main patients characteristics are detailed [N=147, M/F:75/72, 111 in early stage disease, Del17p in 11 patients and Del11q in 15]. Median age was 422 | haematologica | 2017; 102(s2)
69 years (range 34 to 86). Ninety patients were untreated during the follow-up period. Median follow-up duration was 30 months (range 0 to 101). Furthermore, sFLC measurement was assessed in 57 patients who progressed during the study and required treatment according to international guidelines. ROC curve analysis determines cut-off level of K/L ratio at 1.88. Abnormal sFLC was observed at diagnosis in 50.9% (N=29) of all treated patients. The mean ± SD ratio of sFLC in the untreated patients group and in the treated patients group was 1.51±2.08 and 2.80±3.75 respectively (p=0.0082). Considering the sFLC levels (kappa + lambda), the mean±SD in the untreated patients group and in the treated patients group was 29.1±17 and 53.0±11.9 respectively (p<0.0001).

Treatment systematically induced a modification of the sFLC K/L ratio. Interestingly, the fraction of patients who had the lowest ratio of an abnormal k/l ratio was associated with positive MRD determined by FCM with 82% specificity and a 95% positive predictive value. Moreover, median time to treatment income for patients in early stage disease with ratio >1.88 was 12 months while it is not reached in those with ratio ≤1.88 (p<0.0001) (figure 1).

Summary/Conclusions: In our study we observed that sFLC K/L ratio determination as a technically simple, standardized and cost-effective test to evaluate new prognostic factors. Moreover, we found a relationship between HYPO and a risk of more than 40%. There was no difference in dense- or alpha-granule release between the patient groups, and these indicators remained in their normal ranges. There were also significant differences in aggregation assay with ADP (25±16% versus 36±18% for bleeding and non-bleeding patients, p<0.001), collagen (38±19% versus 53±20%, p=0.001), and ristocetin (53±22% versus 62±20%, p=0.02). Interestingly, the patients with bleeding had negative correlation with platelet aggregation with a lower mean platelet count that those without (120 versus 170 thousands per microliter, P<0.0001) and higher lymphocytosis (74 thousands per microliter, P<0.0001) 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 (lg).

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 (lg) levels (e.g. IgG, IgM and IgA) at diagnosis and calculated CLL-IPI. HYPO was defined as having on our laboratory cut-offs (IgG 70mg/dl, IgG 700mg/dl in 22%, and IgM lower 40mg/dl in 33.7%). Forty-six percent of patients presented deficit of at least one lg class, while 7.8% of patients had all lg low. Each lg 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). However, no recognized prognostic/predictive lg cut off has been reported to date, we aimed to identify a prognostic threshold for each lg class.

Results: From 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 lg class, while 7.8% of patients had all lg low. Each lg 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). However, only IgA deficit maintains statistical significance in multivariate analysis [HR 1.58 (1.08-2.35)]. A prognostic threshold for each lg class was identified maximizing the differences in TTFT and the following values were obtained: 80mg/dl for IgA, 410mg/dl for IgG and 186mg/dl for IgM (Figure 1, A-B-C). Considering CLL-IPI, 19 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-off. Moreover, we found a relationship among CLL-IPI and both IgA and IgM values, using when the newly validated lg cut-off. Finally, CLL-IPI was a stronger prognostic factor for TTFT than HYPO in our analysis. However, the addition of IgA deficit to CLL-IPI appears to further improve CLL prognostication.

Summary/Conclusions: In conclusion, HYPO significantly impacts on CLL prognosis. Moreover, even if CLL-IPI has a stronger prognostic value for TTFT compared to HYPO, the addition of IgA deficit appears to further improve CLL prognostication.
E1031
CLL: IS LYMPHOCYTE DOUBLING TIME (LDT) A RELEVANT PROGNOSTIC PARAMETER IN THE ERA OF PROGNOSTIC BIOMARKERS?
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Background: In CLL, tumor doubling time is reflected by the pace at which lymphocytes increase in blood (lymphocyte doubling time or LDT). However, since LDT is rarely available at the time of diagnosis, its role in assessing prognosis in patients in CLL is controversial.

Aims: To reassess the prognostic significance of LDT in a large series of patients.

Methods: Retrospective single-center study based on 629 patients diagnosed with CLL/SLL. LDT was measured at the time of diagnosis if prior WBC counts were available or calculated after diagnosis by linear regression analysis, usually over a treatment-free period of 2 months and including at least three WBC counts.

Results: 140 patients displayed short LDT (≤12 months) and 489 long LDT (>12 months). The median follow-up was 13.4 years (6.1-22.5) and 11.2 years (2.3-30.9), respectively. Patients with short LDT were younger (p=0.005), had more advanced clinical stage (p=0.001), higher ALC (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 more frequently treated with alkylating agents than purine analogues. Furthermore, there was no significant differences in response rates (ORR 59% with 29% CR vs 69% with 29% CR; p=0.253). Overall survival (OS) was shorter in the group with short LDT (median: 7.2 vs 12.2 years; p<0.001). Univariate analysis demonstrated a significant correlation between OS and advanced clinical stage, age >70 years (HR 2.0 (95% CI: 1.0-3.9), p=0.028) and high-risk FISH genetics (del17p, del11q) (all p<0.001). Likewise, mutations in NOTCH1 (p<0.001), SF3B1 (p=0.027), ATM (p=0.028) and TP53 (p=0.028) were associated with OS. In a multivariate analysis including clinical stage, age, LDT, IGHV, ZAP70, FISH cytogenetics and ATM or TP53 (type 1, 2, 3), short LDT increased the risk of progression (p=0.035) and death (p<0.001).

Summary/Conclusions: This study shows that LDT continues being an independent prognostic parameter for OS in the era of biomarkers. In contrast, LDT did not correlate with response to therapy and, accordingly, cannot be regarded as a response predictor to chemo(immuno)therapy. Finally, LDT warrants investigation in the setting of novel therapies.

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/unmutated IGHV (IGHV6-2-4 (95% CI: 1.5-4.0), p=0.003), and presence of TP53 mutation (HR 2.0 (95% CI: 1.3-9.3), p=0.041).

Summary/Conclusions: This study shows that LDT continues being an independent prognostic parameter for OS in the era of biomarkers. In contrast, LDT did not correlate with response to therapy and, accordingly, cannot be regarded as a response predictor to chemo(immuno)therapy. Finally, LDT warrants investigation in the setting of novel therapies.

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 17q region containing its locus, is a well-established marker of poor prognosis and chemoresistance to traditional chemotherapeutic agents. Fluorescence in situ hybridization (FISH) is a useful tool for the detection of the deletion. Nevertheless, its sensitivity is influenced by the number of blood-cell lineages that carry the aberration, the absolute count of deletion-positive cells, and the proportion of deletion-positive neoplastic cells relative to deletion-negative neoplastic cells and non-neoplastic cells, in the whole blood or bone marrow sample. The latter issue can be minimized by purifying the sample through the selection and separation of tumor cells, using techniques such as fluorescence-activated cell sorting (FACS).

Aims: In this study, we aim to evaluate the benefit of using purified samples of neoplastic CLL lymphocytes for the detection of TP53-deletion by FISH, when compared to full samples.
Methods: We reviewed all CLL samples that were submitted for the investigation of TP53-deletion through FISH, in our Lab, between January 1st 2011 and February 28th 2017. Results obtained on tests performed on whole mixed cellularity samples were compared with results obtained directly in FACS purified CLL clonal lymphocytes. 

Results: We analyzed 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 neoplastic cells was only introduced within the last two years of the study period, it accounted for 39.0% of all tested samples. This poor prognostic aberration was identified in 15.8% of patients in the overall cohort, with no differences in the incidence of a positive finding between mixed cellularity samples and FACS purified samples (15.6% vs 16.2%, respectively, p=NS). In contrast, the average proportion of positive cells within a positive sample was markedly different between mixed cellularity samples and FACS-processed samples, increasing nearly three-fold through the purification of the sample, from 24.0±15.9% to 62.9±33.3%, p<0.001. In fact, in 57.7% of all patients who were tested after FACS separation of CLL cells, the TP53-deleted clone was larger than 50% of neoplastic clonal lymphocytes, making it the primary clone.

Summary/Conclusions: We observed that the pre-processing of the sample through the FACS-supported purification of CLL neoplastic lymphocytes revealed that the TP53-deleted clone was nearly three-fold larger than suggested by the mixed cellularity sample, increasing from an average of a quarter of all cells, to nearly two-thirds. This finding uncovered that the TP53-clone was, in fact, the primary major clone within the neoplastic lymphocyte population in the majority of patients. Considering the poor prognosis conferred by the aberration, and its impact on current treatment decisions, it is quite significant to correctly identify a primary deletion-positive clone, instead of mislabeling it as a secondary minor clone.

E1034

PRIMARY PEFGILGRASTIM PROPHYLAXIS VERSUS FILGRASTIM GIVEN "ON DEMAND" FOR CLADRIBINE - INDUCED NEUTROPENIA IN Hairy Cell leukemia


Background: Most targeted therapies in the management of chronic lymphocytic leukemia (CLL) lead to high overall response rates but complete remissions are rare. Achieving complete remission (CR) is associated with improved clinical outcomes such as longer time to progression; however little is known about the economic benefits associated with achieving CR.

Aims: The objective of the study was to compare healthcare resource utilization among CLL patients initiated on first-line treatment who achieved CR versus those who did not.

Methods: This was a retrospective chart review study. From July to August 2016, 93 US oncologists/hematologists provided data abstracted from medical charts of their CLL patients who initiated a first-line CLL treatment between January 2010 and December 2014. The study collected patient demographics, clinical characteristics, response to first-line therapy, and the number of all-cause hospitalizations between first-line therapy initiation and end of the data follow-up (i.e., patient’s date of death, end of care, or data collection date, whichever occurred first). Patients were selected based on their best response to first-line therapy (i.e., CR, partial remission [PR], stable disease [SD] and progressive disease [PD]) as defined by the physician according to iwCLL 2008 criteria. The targeted number of patients in each category was a priori determined based on rates of response observed in clinical trials. The incidence of all-cause hospitalization was compared between patients who achieved CR and those who did not (including patients with PR, SD or PD) using univariate and multivariate generalized linear models with a Poisson distribution. As patients had different follow-up, incidence rates were reported per-patient-per-month (PPPM). Multivariate regression models were adjusted for age, gender, selected comorbid conditions, time from CLL diagnosis to first-line initiation, and Eastern Cooperative Oncology Group (ECOG) status.

Table 1.

Results: Patient-level data was collected for 179 patients who achieved CR and 151 patients who did not achieve CR (120 patients with PR, 25 with SD, and 6 with PD). Average time from CLL diagnosis to first-line initiation was 8.4 months for patients who achieved CR and 13.3 months for those who did not. The majority of patients were male (65%), the average age was 63 years, and 80% of patients had an ECOG of 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=4.9, p<0.05).

Summary/Conclusions: Results from this study showed that achieving CR to first-line therapy (vs. not achieving CR) is associated with reduced frequency of all-cause hospitalizations. This suggests that, in addition to the clinical benefit associated with CR achievement, treatment strategies in CLL that improve CR may help reducing the economic burden of CLL management for both patients and payers.

E1036
RITUXIMAB (R) USED AS A SINGLE AGENT FOR AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) IN TREATMENT NAÏVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS INDUCES ALSO SIGNIFICANT DISEASE RESPONSE WITHOUT TOXICITY


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Background: There are very few effective treatment options for steroid refractory AIHA of CLL or for CLL patients(pts) that are unable to receive corticosteroids. R has been noted to be active in certain autoimmune hematologic disorders while experience with single-agent R in untreated CLL pts is very limited.

Aims: To report our experience concerning the use of R as a treatment of AIHA occurring during the first course of treatment of naive/CLL pts by analyzing concomitantly its efficacy and safety as a single agent in CLL therapy

Methods: 15 pts diagnosed with CLL who received R due to AIHA were included in this study. Staging was performed at diagnosis (Binet system). Pts were placed on R at the standard dose I.V. of 375mg/m2 once weekly for 6 consecutive weeks because of contraindication of corticosteroids administration.

Results: Pts’ median age was 60 (range, 42-83 y). (8 out of 15, males), 10 having disease stage A and 5 B. Two were presented with splenomegaly and 1 with B-symptoms. 12 pts (83%) had leukemoid lymphocyte counts of more than 50x10^9/L. Median time from diagnosis, the AIHA diagnosis and to 1st R infusion was 59 mos. All 15 pts completed the 6-week course of R and were assessable for response. The median WBC and the median absolute lymphocyte count(ALC) before R administration and after the end of 6-week course are shown in the Table. Resolution of the AIHA effect was achieved in all pts whereas in 4 there was a persistence of positive DAT without evidence of active hemolysis. After the 6 weekly R infusion 13 out of 15 pts (86.6%) showed also disease response. 12 pts experienced PR (80%) and 1 CR (6%). All pts with advanced disease also responded entering PR. Resolution of splenomegaly was documented in both splenoegalnic 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 pt with WBC>40x10^9/L without need for hospitalization. None of the pts experienced severe tumor lysis syndrome, pulmonary insufficiency, myelosuppression or opportunistic infections.

Summary/Conclusions: A) R is an effective agent for AIHA treatment with concomitant significant activity against CLL and therefore could be the standard of care for CLL pts with AIHA, especially for the cohort of pts with comorbidities. B) We confirm previous data that: 1) single-agent R induces significant responses in treatment naïve CLL pts 2) R is well tolerated and its administration is not associated with myelosuppression or immunosuppression 3) R as a single agent could be an excellent first-line treatment option for pts who are very elderly or who have a poor performance status

E1037
ATTAINMENT OF COMPLETE REMISSION IS SIGNIFICANTLY ASSOCIATED WITH 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 a 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, 17p deletion and/or TP53 mutation) are less likely to achieve deep responses and experience poorer outcomes. Therefore, less is known about the relationship between CR and survival outcomes in R/R CLL pts.

Aims: To quantify this association, we generated meta-analytic estimates of PFS and OS reported in clinical trials using the proportion of study patients with CR as a predictor variable.

Methods: We performed a systematic literature review of PubMed/EMBASE up to Nov 2014 and congress abstracts 2012‒2014. Randomized controlled trials and observational studies evaluating any treatment in R/R CLL pts were eligible for inclusion. Data were extracted from publications as median survival, the proportions of pts surviving at specific follow-up times, or individual event occurrence. Pooled estimates were calculated using random-effects models and the DerSimonian-Laird method. Response variables included studies that were not also associated with CR, our variable of interest. The hazard ratio (HR; and 95% credible interval, the Bayesian analog to confidence intervals) of survival for each 10% increase in CR among a population was estimated to be 0.94 (0.86, 0.99). The median OS for hypothetical populations with 0% CR, 25% CR, or 50% CR were 22.9 mo, 44.7 mo, and 61.9 mo. Corresponding median PFS estimates were 10.0 mo, 21.9 mo, and 30.3 mo. (Figure 1).

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 predictive 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.
APLICATION OF THE CLL-IPI AND THE MDACC PROGNOSTIC INDEXES IN A LOCAL COHORT OF CLL PATIENTS

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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, β2-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, 79 as intermediate risk, 46 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, β2-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).

CHRONIC LYMPHOCYTIC LEUKEMIA: PROGNOSTIC VALUE OF CLINICAL STAGES AND CLASSICAL PROGNOSTIC PARAMETERS DEPENDING ON TREATMENT MODALITY

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Background: Prognostication is a key component in the management of patients with chronic lymphocytic leukemia (CLL). Prognostic factors however may change as a result of the introduction of more effective therapies.

Aims: To investigate whether the prognostic value of classical parameters has changed over time.

Methods: Retrospective single-center study of prognostic factors and outcome in patients with CLL diagnosed before (n=454) and after (n=903) 1995 when purine analogs and subsequently chemoimmunotherapy (CIT) were introduced in CLL treatment at the Hospital Clinic, Barcelona.

Results: The median follow-up was 8.3 years (0.1-33.0) for the overall series and 24.9 years (21.9-33.0) and 7.8 years (0.1-21.3) for patients diagnosed before and after 1995, respectively. Patients diagnosed before 1995 were older (p=0.001), had more advanced clinical stage (p=0.001), higher absolute lymphocyte count (ALC) (p=0.001), shorter LDT (p=0.001), and more often anemia (p=0.001) and thrombocytopenia (p=0.001) and increased serum LDH levels (p=0.019) than those diagnosed thereafter. There were no differences in β2-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% (p=0.08) vs 45% (p=0.08)]. The type of therapy given to patients diagnosed before and after 1995 was: alkylating agents (91% vs 89%, p=0.1), CIT (75% vs 81%, p=0.3), and more often immunotherapy (both p=0.028). The proportion of patients achieving complete response (CR) and partial response (PR) was higher in the period after 1995 (p=0.001) and 1995 (p<0.001) for PR and CR (both p=0.001), respectively.

Secondary endpoints were time to therapeutic failure, time to progression, progression-free survival (PFS), time to relapse or death after CR or PR, quality-of-life (EQ-5D questionnaire), and frequency of adverse drug reactions (ADR). Secondary endpoints were similar in both periods.

Results: Of the 196 patients who enrolled between June 2012 and August 2015, 29 patients were included in the Safety Population, 244 in the Full Analysis Set (FAS; patients in the Safety Population who had ≥1 response evaluation). Most patients in the FAS were male (59.7%); mean age was 61.5±8.9 years. Overall, 35.6% of patients were ≥65 yr old and 80.5% had ≥1 comorbidity such as decreased renal function. The ORR was 83.2%; CR and PR rates were 59.7% and 23.5%, respectively. Generally, response rates were slightly higher than those reported in the Phase 3 pivotal trial (Knauf et al. J Clin Oncol. 2009). Eradication of minimal residual disease was achieved in 23 of the 84 evaluable
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-SD 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 2 (%)</th>
<th>Grade 3 (%)</th>
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<tbody>
<tr>
<td>Anemia</td>
<td>45.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>21.7</td>
<td>17.6</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2.3</td>
<td>0.5</td>
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**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.

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**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 DEPENDENT 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 sub-clones 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 sub-clones 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 GADD45A whose deficiency is associated with mutagenesis (Hollandcr 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 associated 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 follow-up of 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, TPS3) or downregulated (BAD, BID, MCL2 NOTCH3, PDKPK1) 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.

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**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:** CD26 expression in peripheral blood of patients with chronic myeloid leukemia (CML) is associated with the disease and disease status. However, the methods used for CD26 detection are not simple and rapid enough to be applied in the clinical practice.

**Aims:** We developed and validated a new technique allowing simple and rapid detection of CML stem cells in peripheral blood of CML patients.

**Methods:** We used human peripheral blood obtained from patients with CML in chronic phase (CP), accelerated phase (AP), and blast crisis (BC) and healthy controls. The cells were stained with a FITC-conjugated anti-CD26 antibody and analyzed by flow cytometry. The sensitivity and specificity of the method were determined by comparing the results with those obtained by the standard method (immunochemistry).

**Results:** The new method allowed the detection of CD26+ cells with high sensitivity and specificity. The median percentage of CD26+ cells in CP, AP, and BC was 10.5%, 12.3%, and 15.2%, respectively. The median percentage of CD26+ cells in healthy controls was 4.2%.

**Conclusion:** The new flow cytometry method is a simple and rapid tool for the detection of CML stem cells in peripheral blood, which can be easily applied in the clinical practice.
Aims: We investigated accuracy and specificity of flow cytometry PB cytometry also in PB during treatment with tyrosine kinase inhibitors.

Methods: Pts 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−/Lin− stem cell fraction and CD26 appeared a robust biomarker for identifying CML LSCs within the normal BM compartment. We recently demonstrated that CD34+/CD38+/CD26+ LSCs can be easily identified by flow-cytometry also in PB during treatment with tyrosine kinase inhibitors.

Aims: We investigated accuracy and specificity of flow cytometry PB CD34+/CD38−/Lin− LSCs identification as a new tool for the diagnosis of CML.

Results: PB samples from 107 pts with myeloproliferative features were evaluated for CD26+LSCs. Leucocytes median value was 52x10^9/L (range 5-409x10^9/L). In 83/107 (77.5%) pts we showed CD34+/CD38−/Lin− LSCs in PB and in 83/93 (100%) the diagnosis of CML was confirmed by cytogenetic, FISH and/or BCR-ABL1 RT-PCR analysis. Median value of circulating PB CD26/LuL 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 (range 0,27-698) and a positive correlation with leukocyte count (p<0.01) was found. Median value of circulating PB CD26/LuL 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 (range 0,27-698) and a positive correlation with leukocyte count (p<0.01) was found.

Summary/Conclusions: The prospective study aims to confirm the possible role of genetic predisposition and behavior of specific pro/anti-inflammatory biochemical parameters in the atherosclerotic pathogenesis during TKIs treatment. Multicentric “Prospective study of TKI induce pro-atherothrombotic status in CML, KIARO study” (Grant support: AIRC-IT) including Chronic Phase CML patients treated with any first line approved TKI in which clinical, genetic and biochemical pro-atherothrombotic profiles were evaluated at diagnosis and during treatment.

Aims: This prospective study aims to confirm the possible role of genetic predisposition and behavior of specific pro/anti-inflammatory biochemical parameters in the atherosclerotic pathogenesis during TKIs treatment.

Methods: Enrolled pts were prospectively evaluated for presence of traditional CVRFs, atherothrombotic episodes, presence of Single Nucleotide Polymorphisms (SNPs) associated to CV risk (Cardiokit) and plasma levels of several pro and anti-inflammatory cytokines. In this first interim analysis we focused on levels of LDL, oxidized-LDL (oxLDL), TNFα, IL-6 and IL-10 and the presence of SNPs of LDL-R (rs1122608), LOX-1 (rs3736235), and IL-10 (rs1800896) genes.

Results: 12 Italian Hematology Units participated to the study and up to date 95 CML patients were enrolled. We here report data from the first 43 patients on TKI treatment for at least 12 months (15 nilotinib, 14 imatinib and 14 dasatinib). No CV events were recorded to date. At diagnosis, levels of LDL (143.5±13.2), ox-LDL (237.4±99.5), TNFα (3.91±2.51), IL-6 (1.96±0.99) and IL-10 (0.34±0.15) were evaluated for the whole cohort and according to the presence/absence of the detrimental G/G allele of LDL-R (H.R. 2.26, p<0.01), IL-10 (H.R. 1.85, p<0.05) polymorphisms. During TKIs treatment we observed increased levels of LDL (p<0.05) and oxLDL (p<0.05) only in the nilotinib cohort at 3 and 12 months of treatment, regardless of the concomitant use of CV medications. No differences in TNFα and IL6 levels during the first 12 months of treatment were observed between basal levels of LDL, oxLDL and IL10 with the presence/absence of the detrimental G/G allele of LDL-R (H.R. 2.26, p<0.01), IL-10 (H.R. 1.85, p<0.05) polymorphisms. During TKIs treatment we observed increased levels of LDL (p<0.05) and oxLDL (p<0.05) only in the nilotinib cohort at 3 and 12 months of treatment, regardless of the concomitant use of CV medications. No differences in TNFα and IL6 levels during the first 12 months of treatment were observed.

Summary/Conclusions: This interim analysis, although still very preliminary, suggests that in nilotinib patients the high levels of LDL and oxLDL in combination with low levels of IL10, could induce a persistent pro-inflammatory/oxidative status potentially favoring atherothrombotic events. Additional biochemical and genetic data as well as prolonged clinical observation are needed to confirm this hypothesis. Patients enrolment and monitoring is ongoing.
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 is an adverse event after TKI cessation which 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. EVs were isolated from peripheral blood by ultracentrifugation. EV-miRNA expression profiling was performed on the EVs using NGS. The expression values of each miRNA for each sample were compared to the healthy controls. This latter T-UCR (uc.145) was associated with development of TKI resistant CML cells. This latter T-UCR (uc.145) was associated with development of TKI resistant CML cells.

**Results:** Peripheral blood samples from 45 CML patients and 15 healthy controls. This latter T-UCR (uc.145) was associated with development of TKI resistant CML cells. This latter T-UCR (uc.145) was associated with development of TKI resistant CML cells.

**Summary/Conclusions:** CML patients with increased EV-mir-140-3p achieved levels similar to those of healthy controls after re-initiation of TKI therapy, whereas CML patients who did not experience musculoskeletal pain had increased EV-mir-140-3p expression. These observations suggest that EV-mir-140-3p is a potential biomarker for the development of musculoskeletal pain during TKI discontinuation in CML patients.

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E1047

**SOLUBLE AND MEMBRANE-BOUND RECEPTOR–LIGAND IMMUNE CHECKPOINTS AND CHRONIC MYELOID LEUKEMIA: CORRELATIONS WITH THE MUSCULOSKELETAL PAIN SYNDROME**

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**Background:** Chronic myeloid leukemia (CML) is a hematopoietic malignancy that affects the white blood cells and the bone marrow. It is caused by an abnormality in the karyotype of certain chromosomes, leading to the production of an abnormal protein called BCR-ABL. Although the disease can be treated with tyrosine kinase inhibitors (TKIs), there is a high risk of developing adverse events, including musculoskeletal pain. The musculoskeletal pain syndrome is a common adverse event in CML patients treated with TKIs, and its mechanisms are not fully understood.

**Aims:** This study aimed at the analysis of lymphocyte subsets expression and the plasma levels of immune checkpoint inhibitors during chronic myeloid leukemia (CML) therapy. We aimed to investigate the potential role of immune checkpoints in CML patients with musculoskeletal pain, and to understand the mechanisms underlying the development of this syndrome.

**Methods:** We analyzed peripheral blood samples from 45 CML patients and 15 healthy controls. We used multi-parametric flow cytometry to analyze the expression of lymphocyte subsets and the levels of immune checkpoint inhibitors. The plasma levels of the immune checkpoint inhibitors were measured using ELISA.

**Results:** We found that the expression of immune checkpoint inhibitors was significantly higher in CML patients with musculoskeletal pain compared to healthy controls. The most relevant results were the increased expression of PD-L1 and PD-L2 in CML patients with musculoskeletal pain. These findings suggest that immune checkpoint inhibitors play a role in the development of musculoskeletal pain in CML patients.

**Summary/Conclusions:** Our results indicate that the musculoskeletal pain syndrome in CML patients is associated with the activation of immune checkpoints, which are known to modulate the immune response. This suggests that targeting immune checkpoints could be a potential therapeutic strategy for the management of musculoskeletal pain in CML patients.
E1048
TYROSINE KINASE INHIBITORS SIGNIFICANTLY CHANGE THE EXPRESSION OF POLYCOMB GENES IN CHRONIC MYELOID LEUKEMIA

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).

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, SCLM2, CBX2) were down-regulated in at least half of samples. The expression of 5% of PcGs was “mixed”, up- or down-regulated in different samples. Among the up-regulated genes, some could be relevant from a biological point of view: 1) HLTF, a target for RUNX1, whose low expression in acute leukemia is correlated with poor outcome; 2) PHC2, able to silence the HOX genes, overcoming the multidrug resistance in myeloid models; 3) PCGF5, that is a marker of normal hematopoiesis; 4) MOV10, that has been reported to have an anti-viral activity, increasing levels of gamma interferon. This up-regulation is particularly interesting, because concerns all assessed samples and could explain our previous observation that Torque Teno virus replication does not occur in CML patients during TKIs therapy; 5) in the only “warning” patient, the up-regulation of SIRT2 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) ZBTB51, 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 seem to be relevant from a biological point of view: 1) CBX2, that binds 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) ZBTB51, 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 seem to be relevant from a biological point of view: 1) CBX2, that binds 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) ZBTB51, 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 seem to be relevant from a biological point of view: 1) CBX2, that binds 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) ZBTB51, 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 seem to be relevant from a biological point of view: 1) CBX2, that binds 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) ZBTB51, 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 seem to be relevant from a biological point of view: 1) CBX2, that binds 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) ZBTB51, 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 seem to be relevant from a biological point of view: 

Summary/Conclusions: We demonstrated that PcGs de-regulation occurs in CML patients during the treatment with TKIs, with possible pathogenetic implications. Hugger 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

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=15) were segregated as responders (n=10) and failures (n=8) as per ELN, 2013 guidelines. Healthy controls (n=5) were also enrolled. DNA from blood of subjects was subjected Next Generation Sequencing (NGS). Mutations present in one patient group and absent in opposite group were considered as prognostic markers, whereas rare mutations, present in more than 50% of enrolled patients and absent in healthy controls, were considered as susceptibility markers

Results: We discovered mutations in genes associated with cancer or cancer related functions in different patient groups as markers. Five of them: rs116201358, rs17882014, rs4014996, rs52897880 and rs2274329 in CBA, HLA-DRB1, UNC93B1, APOH and CA6 genes respectively, were present in responders; rs4594 in MFGE8 was present in failures. Mutations in HLA-DRA1 (rs17878951, rs11554462, c.239C>G), HLA-DRB5 (rs137863146), RPHN2 (rs193173933), CYP2F1 (rs116958558), KCNJ12 (rs76684759), FUT3 (rs151218854), BM1C (rs28370522) and PRSS1 (rs144422014) were present in half of responders.

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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Figure 1.
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 cytochromes ensure homeostasis at the cellular and tissue level, carrying out the metabolism of toxic compounds that can damage the genome of the cells. Previous studies have shown that there are genetic polymorphisms of these genes in a number of neoplastic diseases, including leukemia. In individuals with weakened functional genotypes of A2425G polymorphism of CYP1A1 gene expression of this enzyme and, consequently, inactivation of xenobiotics must occur very slowly, thus creating conditions for adverse action of harmful metabolites in the genome of the cells. Currently, the scientific literature discus ses the role of the negative allele A2425G polymorphism of CYP1A1 gene in the development of hematological malignancies. However, the adverse roles of genotypic variants for this gene in oncogenesis of BCR-ABL-positive patients with CML have studied not enough.

Aims: Evaluation the role of A2425G polymorphism of CYP1A1 gene in the formation the mutant clone of tumor and development of CML.

Methods: The work is performed on DNA samples isolated from the peripheral blood of the patients in the clinic of scientific research Institute of Hematology and blood transfusion in Uzbekistan. We studied 146 patients with CML. The control group was formed from 217 individuals of Uzbek nationality, without any cancer disease. The diagnosis of CML verified in accordance with the International nomenclature ISCN. Standardized PCR with detection in real-time was carried out on a thermal cycler Rotor-Gene 6000 (Corbett Research, Australia), using a set of reagents "RealTime Ready" and primers of "ligase Chain Reaction" (InterLabServis, Russia). Testing A2425 polymorphism of CYP1A1 gene was performed on a programmable thermal cycler of the company "Applied Biosystems" (USA) using test systems company "LifeTech" (Russia) according to the manufacturer’s instructions. Statistical analysis of results was carried out using the statistical software package "2009 OpenEpi, Version 2.3.1." Results: The frequencies of allele A and G are as follows: 76.7% 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 in patients with CML 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 1.1-3.6) and patients without CML. At the same time, differences in the results did not reach statistical significance (P>0.05). Functions of allele A and G were as follows: 76.7% and 12.3% in CML patients, and 93.3% and 6.7% in the control group (21.9% vs 13.4%, respectively). The risk of mutant formation of the tumor clone in carriers of this genotype was 1.4 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 A/A 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 1.4 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 A/A of A2425G polymorphism of CYP1A1 gene has a protective character in relation to risk of CML.

Summary/Conclusions: Our results suggest that the G allele and the heterozygous genotype A/G A2425G polymorphism of CYP1A1 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 CYP1A1 gene has a protective character in relationship to risk of CML.

Figure 1. Evolution of hematologic toxicity grade 3-4 with time (all treatments sequences included).

Results: Demographics, risk and treatment distribution: 893 patients (533 men, 360 women) with a median age at diagnosis of 52 y (14-94y) were included with a follow up of 85±7 months (m) from diagnosis, 78±6.6 m from first treatment, and 69±6 m from first TKIs. 151 patients (16,9%) were over 70y. The risk distributions were as follows: Sokal: low (L) 48%, intermediate (I) 37% and high (H) 14%; Euro score: L 50%, I 45% and H 5%; EUTOS L: 89% 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
Poitiers, France, 4Winship Cancer Institute at Emory University, Atlanta, United States, 3Inserm CIC 1402, CHU de Poitiers, patients on IM, whether or not they had dose reductions for an AE; these rates Patients on DAS maintained higher molecular response rates than Table 1.

1Universitätsklinikum Jena, Jena, Germany, 2University of Texas MD Anderson in DAS- and imatinib (IM)-treated patients with dose reductions or interruptions that this probability is not different across different sequential treatments (imatinib 1st line or post-IFN, or switched to 2ªGTKis due to intolerance or failure) (1). This fact emphasizes the rescue potential of available TKI therapies. 2. Hematologic toxicity grade III-IV in the first two years identified a group of patients with worse survival outcome. 3. Patients over 70 years have shorter survival due to reasons different than progression. 4 Second GTKis showed better hematologic toxicity profile.

Reference

E1052
5-YEAR EFFICACY OF DASATINIB AND IMATINIB IN NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) WITH DOSE MODIFICATIONS FROM DASISION A. Hochhausa1,1, E. Jabbrob2, H. Kantarjian2, F. Guilhot3, V. Kotas2, T. P. Hughes3, S. Shelefa3, L. Lif2, J.E. Cortesa3, 1Universitätsklinikum Jena, Jena, Germany, 2University of Texas MD Anderson Cancer Center, Houston, United States, 3Inserm CIC 1402, CHU de Poitiers, Poitiers, France, 4Winship Cancer Institute at Emory University, Atlanta, United States, 5SAHMRI, University of Adelaide, Adelaide, Australia, 6Bristol-Myers Squibb, Princeton, United States

Background: Multiple dosage strengths are approved for dasatinib (DAS), permitting dose-optimization strategies for patients who experience adverse events (AEs). In a 2-year retrospective analysis of DASISION, efficacy was maintained in DAS- and imatinib (IM)-treated patients with dose reductions or interruptions to manage AEs (Jabbour ASH 2011); cytogenetic and molecular response rates were higher for patients given DAS vs IM, even when daily doses were modified. Longer term follow-up is needed to fully understand the potential impact of dose reductions on efficacy.

Aims: To evaluate the effect of dose reduction for any AE and for pleural effusion on efficacy in DAS- or IM-treated patients from DASISION.

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-
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=0.100 (1.000-2.002), P=0.004] and ABBG2 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=0.101 (1.000-2.002), P=0.001] and UGT1A1 *1/*1 [hazard ratio=0.475 (0.246-0.919), P=0.027] were independent factors for the elevated total bilirubin.

Summary/Conclusions: In this study, we identified the relationship between NIL trough concentration and liver dysfunction. Our finding suggests that therapeutic drug monitoring might help avoid drug interruption and discontinuation because of AEs, especially liver dysfunction.

E1054

VERY EARLY MOLECULAR RESPONSE (VEMR) WITH FRONTLINE DASATINIB TREATMENT IS A STRONG PREDICTOR OF LONG-TERM BCR-ABL1 TRANScript LEVELS IN CHRONIC MYELOID LEUKEMIA PATIENTS: PCR-DEPTH STUDY


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Background: In BCR-ABL1 tyrosine kinase inhibitor (TKI) treated chronic phase chronic myeloid leukemia (CP-CML), early molecular response (EMR) at 3 months is currently identified as being one of the most important prognostic factors. Sokal risk score and dose intensity during first 3 months were strongly associated with achievement of EMR. As dasatinib is a novel, oral tyrosine kinase inhibitor with improved potency, identification of very early molecular response (VEMR) would be beneficial.

Aims: We evaluated the possibility of the VEMR at 1 month predicting long-term outcomes in newly diagnosed CP-CML patients treated with dasatinib.

Methods: In this 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 months, 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%) out of 102 patients were still on dasatinib treatment and 22 patients discontinued treatment (12 events or failure (n=2) or adverse events (n=8) or other reasons (n=9)). The BCR-ABL1 mutations, assessed in 10 patients after dasatinib discontinuation, were detected in 3 patients which were all T315I mutation. The cumulative CMR by 18 months and MMR by 24 months were 20.5% and 79.6% respectively. In safety analyses, grade 3/4 thrombocytopenia (30.3%) was most common. Pleural effusion occurred in sixteen (15.6%) patients which were mostly grade 1/2. The cut-off value of BCR-ABL1 transcript on Day+28 was 40% by ROC curve analysis. Among 95 patients who had available molecular data of both D+28 and 12 months, 50 (62.1%) patients had less than 40% of BCR-ABL1 transcript (VEMR) on Day+28. In 52 (61.2%) patients achieved MMR at 24 months. However, only 27.8% (10 out of 36 patients) of patients without VEMR achieved MMR (p=0.0001). Among 85 patients who had available molecular data of both D+28 and 24 months, fifty two (61.2%) patients achieved VEMR. In 52 VEMR patients, 46 (88.5%) patients achieved MMR at 24 months. However, only 48 (62.1%) patients achieved CMR (p=0.0001). Overall survival (OS) & progression-free survival (PFS) rates by 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).

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-ABL T315I mutation. Overall survival (OS) for 3L CP-CML patients in PACE at 1, 2, 3 and 4 years was estimated to be 91%, 83%, 80%, 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 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.

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) patients bearing a Philadelphia chromosome (Phi+ ALL). Mutations of the BCR-ABL1 kinase domain constitute a major cause of treatment failure in CML and Phi+ALL patients receiving first or second generation tyrosine kinase inhibitor (TKI) treatment. So far, the gold standard procedure to detect BCR-ABL1 kinase domain (KD) mutations is the conventional Sanger Sequencing, endowed with an analytical sensitivity of 15-20%. Recent studies on the implementation of Next Generation Sequencing (NGS) for detection of BCR-ABL1 KD mutations showed a significant dropping down of the sensitivity level (1-5%), improving patient’s treatment management.
Aims: Both NGS and droplet digital PCR (ddPCR) were used in this prospective study. NGS screened all known mutations in the BCR-ABL1 KD and ddPCR targeted only the 3 most common mutations, T315I, E255K and Y253H, which represent approximately 75% of the ABL1 mutations. Patients eligible for the study were i) CML patients with failure or warning to all lines of TKI therapy according to the 2013 ELN-guidelines, with no suspected lack of adherence and ii) Phi+ ALL patients in diagnostic and/or molecular relapse. Monitoring was performed when clinically appropriate.

Methods: Total BCR-ABL1 RNA was transcribed into a long range cDNA covering the kinase and the regulatory and the SH2/SH3 domains of either p190 or p210 BCR-ABL1 transcripts (exons 4 to 10). For NGS, primers designed with the Ampliplex™ Designer Software were selected to generate a set of 10 amplicons. Bar-coded libraries, constructed according to the AmplicSeq™ 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 Phi+ 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 harboured compound mutations: T315I + E255K (6) and T315I + Y253H (3). A high frequency (85%) of T315I, E255K and Y253H mutations was also observed (23/27). As far as these 3 mutations are concerned, reproducibility to determine mutational burden was found to be very high between NGS and ddPCR.

Summary/Conclusions: 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-A2B IN PATIENTS WITH SUBOPTIMAL MOLECULAR RESPONSE ON IMATINIB (NORDUTCHCMLC009)
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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 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) in 2014 (American Heart Association, 2014). Furthermore, in 2014, it was also reported 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. Chronic myeloid leukemia (CML) patients 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, Sultae-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, 3 cases by nilotinib, and 4 cases by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk), Framingham score (2 low, 5 moderate, 7 high risk), and Sultae-score (3 low, 1 intermediate, 4 high risk). There was a significant difference in the rate of IHD between the groups (p=0.01). The numbers of IHD, CI, and PAOD cases were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.78 and 3.34 in the age-
matched general population, respectively. 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, 3 cases by nilotinib, 1 case by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk). Framingham score (3 low, 5 moderate, 7 high risk), and Suita-score (13 low, 1 intermediate, 1 high risk). The incidence rate of IHD and CI per 1000 person-years were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-matched general population, respectively.

Table 1.

**Incidence rate of VAEs**

<table>
<thead>
<tr>
<th>Event</th>
<th>IHD</th>
<th>CI</th>
<th>PAOD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>36,049 events</strong></td>
<td>1,771</td>
<td>8,184</td>
<td>9,184</td>
</tr>
<tr>
<td><strong>Total years</strong></td>
<td>13,200</td>
<td>13,200</td>
<td>13,200</td>
</tr>
<tr>
<td><strong>Incidence rate</strong></td>
<td>1.36%</td>
<td>1.20%</td>
<td>0.69%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The incidence rate of IHD per 1000 person-years were higher in the nilotinib- and lower in the imatinib- and dasatinib-treated CML patients, and the patients showed almost the same rate of CI as compared with the age-matched general population, even though the incidence of VAEs were lower in Japanese compared to the European cohort. More patients were estimated to have very-high and high risk of VAEs in the SCORE and Framingham score assessment tools as compared with the Suita-score tool.

E1059

UPDATE OF CMREGISTRY: AN OBSERVATIONAL, MULTI CENTER, PROSPECTIVE FOLLOW-UP REGISTRY OF PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA WITH A HIGH PROBABILITY OF OBTAINING A DEEP MOLECULAR RESPONSE >CMR4 (IS)

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Background: Since the introduction of Tyrosine Kinase Inhibitors (TKI), many patients diagnosed of Chronic Myeloid Leukemia (CML) in chronic phase achieve a deep molecular response. Around 50% of these patients are expected to manifest a high probability of obtaining a deep molecular response (>CMR4 IS) during their TKI treatment. Several clinical trials are exploring the best way to stop TKI therapy and evaluating patient and disease characteristics that could predict relapse after treatment discontinuation.

Aims: This is an update of the CMRegistry study aimed at collecting clinical data and molecular information from Spanish CML patients that have achieved a series of molecular milestones to any of the tyrosine kinase inhibitors who are likely to achieve, or have already achieved, a deep molecular response (>MR4.0 IS) are included. This likelihood of achieving >MR4 is defined, for the purposes of the study, as a bcrabl/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: 156 patients low risk, 307 intermediate risk and 129 high risk. Eutos classification yielded 714 patients in the low risk and 79 in the high risk categories. The majority of patients received first-line treatment with imatinib (626 patients), dasatinib (39 patients) or nilotinib (87 patients). Of note, 5 patients received bosutinib, 1 patient ponatinib and 74 patients were treated with Interferon previous to TKI administration. So far 14 patients have died of non-CML related conditions such as carcinoma (2 patients), ischemic heart disease, respiratory failure and sepsis. Interestingly, 2 patients developed progression of their CML to accelerated phase and blast crisis (1 patient each) with no deaths. At present, 104 patients (11%) have achieved a MR4.0, 174 patients (18%) a MR4.5 and 123 patients (13%) have obtained a complete molecular remission (undetectable bcr- abl transcripts with a sensitivity of at least 10⁻⁵).

Summary/Conclusions: Almost one thousand CML patients have been included in this spanish prospective study owing to their promising molecular response that would predict for a sustained deep molecular remission. Four hundred and one patients have already achieved a deep molecular response (>MR4 (IS)) and could be enrolled in prospective discontinuation studies.

E1060

ANALYSIS OF DASATINIB AND IMATINIB 5-YEAR EFFICACY AND SAFETY BASED ON BASELINE COMORBIDITY AND AGE IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) IN DASISION

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Background: Patients with CML often have comorbidities, which may influence treatment-related decisions and impact response and survival. In a retrospective analysis of 1-year data from the phase 3 DASISION study, the overall safety or response in dasatinib- or imatinib-treated patients was not substantially impacted by baseline comorbidities, although certain adverse events (AEs) trended higher in patients with ≥1 vs 0 comorbidities (Khoury ASH 2010). Further analysis is warranted to determine how comorbidities may impact long-term outcomes.

Aims: To evaluate the impact of baseline comorbidities and patient age on 5-year safety and efficacy in dasatinib- or imatinib-treated patients from DASISION.

Methods: In DASISION (NCT00481247), patients were randomized to receive dasatinib 100mg/day (N=259) or imatinib 400mg/day (N=260). For this retrospective analysis, patients were grouped as having 0 or ≥1 baseline comorbidity, by baseline disorder (diabetes mellitus, hepatobiliary disease, hyperlipidemia, cardiovascular disorder, or pulmonary condition), or by age group (<46 years, 46–65 years, >65 years). Safety (treatment-related AEs in ≥10% of patients) and efficacy (response rates by 5 years and median times to response) were assessed for each group and treatment.

Table 1.
Results: The number of patients with 0 or ≥1 comorbidity was similar in the dasatinib (66 [25%]; 193 [75%]) and imatinib (67 [26%]; 193 [74%]) arms, respectively; most (>90%) patients were <65 years old. In patients with 0 or ≥1 baseline comorbidity, the median average daily dose was comparable within arms and discontinuation rates (36%-39%) were similar within and across arms (table). The overall safety profiles were comparable in the 0 and ≥1 comorbidity groups in both arms, other than specific AEs, which had a 22 times higher frequency in patients with ≥1 vs 0 comorbidities; the majority of these were grade 1/2 AEs (table). The incidence of peripheral edema increased with patient age for both dasatinib and imatinib (<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 and AEs, though rates were slightly 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 (63 vs 36) or imatinib (MR4.5: 42 vs 47).

Summary/Conclusions: The superior efficacy of dasatinib over imatinib was shown in previous studies. Response rates and times to response were comparable in patients with 0 or ≥1 comorbidity and trended in favor of dasatinib vs imatinib. Although a few AEs (most grade 1/2) appeared to occur at a higher frequency in patients with ≥1 vs 0 comorbidities in either treatment arm, the overall 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 TKI: 36 MONTHS UPDATE OF RADOTINIB PHASE 2 STUDY

Background: Radotinib is an orally active, selective BCR-ABL 1 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 BCR-ABL1 TKIs with a minimum follow-up of 36 months.

Methods: Ph+ CP-CML patients who failed prior TKI therapy were enrolled between July 2009 and November 2011. All patients were treated with radotinib 400mg twice daily. Cytogenetic and molecular assays were performed at 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 (0.3-60.9) months. Cumulative incidence of complete cytogenetic response (CCyR) by 36 months was 90.0% and of patients achieving CCyR, 45.0% (18/40) achieved MMR. The drug-related safety profiles were consistent with those previously reported and new safety issues have not been observed after 12 months. Most drug-related AEs have developed within 12 months, and have shown minimal increase compared with rates at 12 months follow-up. Estimated OS and PFS at 36 months were 87.6% and 85.7%, respectively.

Figure 1. Summary/Conclusions: The 36 months data supports radotinib treatment in TKI failed CP-CML patients maintains the effective response and high rates of OS & PFS rate. Thus, radotinib demonstrated a promising alternative treatment for patients with TKIs failure.
100 YEARS OF CHRONIC MYELOID LEUKEMIA PREVALENCE IN FRANCE

M. Delord 1,2

Background: The success of imatinib and other rationally designed tyrosine kinase inhibitors (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. 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, 25 in 2030 where the tendency inflects, and 30 after 2040. Considering the 100% relative survival hypothesis, a target CML prevalence were defined, the level of which will be nearly reached by 2060. By simulation, we showed that given constant incidence rates and high relative survival hypotheses, the CML prevalence will be driven by population aging, and that the target prevalence, defined as the maximum CML prevalence, should be nearly reached by 2050 to levels above 30 per 100,000 inhabitants.

Figure 1. Summary/Conclusions: Due to high rates of relative survival observed after introduction of imatinib, the trajectory of the CML prevalence in France, as in other western countries, has changed. Given particular hypothesis on the CML incidence rates, this trajectory will bring the CML prevalence by the mid century to levels fully determined by population aging. For France, we have estimated this level above30 cases per 100,000 inhabitants.

The ROLE OF MICRONS IN CHRONIC MYELOID LEUKEMIA THERAPEUTIC SELECTION

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Background: Chronic myeloid leukemia (CML) is characterized by the presence of BCR-ABL fusion gene. This molecular event becomes the main therapeutic target with Imatinib as first-line treatment. In spite of the continued clinical success of Imatinib on CML treatment, the emergence of resistance to tyrosine kinase inhibitors (TKIs) has stimulated the research of the mechanisms involved. These included those related with target changing (e.g. the presence of BCR-ABL gene mutations and amplifications) and with intracellular drug concentrations (e.g. the abnormal levels of influx and efflux transporters such as OCT1/OCCT2 and PgP/BCRP, respectively). MicroRNAs (miRNAs) are important regulators of both mechanisms, and so, could influence TKIs response.

Aims: In this context, we investigated the role of miR-203, miR-21, miR-518c, miR-451 and miR-26 expression levels in TKI response in CML patients, and correlated them with TKI sensitivity, BCR-ABL levels, and disease progression, among other clinical and laboratory data.

Methods: To this end, we assessed the expression levels of miR-203, miR-21, miR-518c, 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-RA). K562-RC cells, generated by continuous exposure to Imatinib, presented an IC50 8x times higher than the parental cell line (K562); in K562-RA 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-518c expression was not detected in any cell line or patient sample. First, we evaluated the miRNAs expression in CML patients. 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-RA compared with sensitive cell line (K562; p<0.05). On the other hand, patients with more BCR-ABL content (between 1.0% and 0.1%) present higher expression of the oncomiRs, miR-21 and miR-26. These miRs were also up-regulated resistant cell lines. MiR-21 was more relevant for K562-RC cells (4-fold higher than K562). LAMA-84 and K562-RA cell lines showed almost 2 times more expression of miR-451. Next, we analyzed if treatment options affected miRs expression. CML patients under Imatinib treatment showed higher levels of miR-451 associated with less expression levels of miR-21 and miR-26. Imatinib had been described to be able to block the BCR-ABL negative feedback on miR-451, increasing miR function. Since miR-21 and miR-26 were also lower expressed, more PTEN is available to block PI3K-AKT-mTOR pathways, decreasing this survival signaling. Opposite profile was observed in patients that changed treatment to a second generation TKI suggesting a different effect of this TKI on microRNA expression.

Summary/Conclusions: Our preliminary results suggested the involvement of miRNAs in BCR-ABL levels regulation and in TKI response, supporting the search of a miRNAs TKI response profile that could predict the response in CML patients. This information could act as powerful tool for the stratification and selection of the best therapeutic approach (lower toxicity and cost effective), contributing to higher survival rates and better quality of life in CML patients.

Work supported by the Faculty of Medicine of the University of Coimbra and Santander Totta Bank, grant reference FMUC-BST-2016-214.

IMPAKT OF ABCB1 AND ABCG2 POLYMORPHISMS ON RESPONSE TO IMATINIB AND 2G-TKIS THERAPY IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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Background: Overexpression of multidrug resistance proteins ABCB1 and ABCG2 confers resistance to anticancer drugs, including tyrosine kinase inhibitors (TKIs). Various ABCB1 and ABCG2 single nucleotide polymorphisms
(SNPs) affect the transporter activity, but their impact on clinical response to imatinib in chronic myeloid leukemia (CML) is discordant; even less is known on their role in patients treated with second generation (2G) TKIs dasatinib and nilotinib.

Aims: To investigate the role of the most common ABCB1 and ABCG2 genetic polymorphism in chronic phase CML patients treated with imatinib and 2G-TKIs.

Methods: We analysed four polymorphisms of ABCB1 (129T>C, 1236C>T, 2677G>T/A and 3435C>T) and two polymorphisms of ABCG2 (34G>A and 421C>A) in 196 CP-CML patients, of whom 139 treated with imatinib (114 in first line and 25 after interferon failure) and 57 treated with dasatinib or nilotinib (22 in first line and 35 after imatinib failure). We compared the rates of optimal response at 3 months (defined as BCR/ABL <10%), at 6 months (BCR/ABL<1%) and at 12 months (BCR/ABL<0.1%), progression-free survival (PFS) and time to treatment failure (TTF) according to the different protein genotypes. TTF was calculated from the start of therapy to any of the followings: progression to accelerated or blastic phase (ABP), death for any cause at any time, and treatment discontinuation for primary or secondary resistance or intolerance. PFS was calculated from the start of TKI to ABP or death.

Results: A total of 196 patients with CP-CML (median age 57 years, range 21-84) were included in the analysis. Frequency of ABCB1 and ABCG2 SNPs expression is summarized in Table 1. Considering response to therapy, either in imatinib-treated patients and in those receiving a 2G-TKI, we did not find any significant difference in terms of optimal response at the various timepoints, TTF or PFS for ABCB1 C1236T, G2677T and C3435T and of ABCG2 G34A and C412A polymorphism, even if there was a trend for a worse PFS in the few patients (n=3) with 1236 allele A treated with imatinib. Conversely, we found a lower rate of optimal response at 3 (p=0.001), 6 (P=0.005) and 12 (p=0.2) months in imatinib-treated patients with TC genotype of ABCB1 T129 SNP, though the small number of patients (7) had probably impact on statistical significance. However, TTF was shorter for ABCB1 129T>C patients, both receiving imatinib (P=0.05) and 2G-TKIs (P=0.07), and also PFS was significantly shorter in this cohort (P=0.003).

Table 1.

<table>
<thead>
<tr>
<th>MDR protein</th>
<th>SNP</th>
<th>Genotype</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>ABCB1</td>
<td>C1236T</td>
<td>CT</td>
<td>32%</td>
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<tr>
<td></td>
<td>G2677T</td>
<td>GG</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>C3435T</td>
<td>GG</td>
<td>34%</td>
</tr>
<tr>
<td>ABCG2</td>
<td>G34A</td>
<td>GA</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>C412A</td>
<td>GA</td>
<td>12%</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.

E1066

THE INTRODUCTION OF SECOND-GENERATION TYROSINE KINASE INHIBITORS MAY REDUCE THE PROGNOSTIC IMPACT OF HIGH-RISK PATIENTS ON patient prognosis as determined by EUTOS score.

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 selected for the study. We classified patients according to EUTOS score in the following periods: 2001-2006 (imatinib era 1G-TKI), 2007-2012 (imatinib era 2G-TKI) and 2013-2016 (next generation TKIs era 3G-TKI). We used Cox regression analysis to determine the cumulative incidence of CML-associated death (CML-ad), progression to accelerated or blastic phase (ABP), treatment discontinuation for primary or secondary resistance or intolerance (TDDR) and event-free survival (EFS). Summary/Conclusions: Among patients considered high-risk according to EUTOS score, the cumulative incidence of CML-associated death, treatment discontinuation and EFS were significantly lower in the next generation TKIs era compared to the imatinib era. 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.
CML and pregnancy had the synchronistic onset of these events (table 1). Sokal nosed in 1st/2nd/3rd trimester in 18/7/6 females correspondingly. Induced abortion patients correspondingly, no data for risk score was in 1 patient. CML was diagnosed low/intermediate/high and EUTOS low/high risk score was in 22/5/3 and 28/2 all 282 pregnancy cases. In certain countries (Russia) up to 21% of women with Ph-positive chronic phase CML during pregnancy. That was 11% of

**Background:**

...characteristics at diagnosis, cytogenetic and molecular parameters, information of therapy, pregnancy outcomes and data of newborns.

**Aims:**

To describe pregnancy outcomes and therapy tactics for CML diagnosed at pregnancy.

**Methods:**

Information regarding CML diagnosed at pregnancy was collected with the participation of countries participating in the observational study of European LeukemiaNet (ELN Pregnancy Registry). The data included CML clinical characteristics at diagnosis, cytogenetic and molecular parameters, information of therapy, pregnancy outcomes and data of newborns.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Table 1. Number of pregnancy cases in CML patients and outcomes of pregnancy diagnosed simultaneously with CML.</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/3rd trimester in 18/7/6 females correspondingly. Induced abortion was done before CML therapy was started in 10 (32%) around 10 obstetrician week (range 6-17), spontaneous abortion happened in 1 woman (at 8th week). Pregnancy was prolonged by consistent desire or religious reasons in 20 females: 2 pregnancies are ongoing now and 18 have ended in childbirth. No treatment till labor was in 5 women. 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 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 9-63) 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 postponed switch from IFN to IM during pregnancy progressed to blast crisis after labor and had furher bone marrow transplant failure while 1 patient after induced abortion developed rapid blast crisis with BCR-ABL compound mutations including T315I.

**Conclusion:**

...the first report of a large database of women diagnosed with CML during pregnancy. Management of this very delicate subset of patients is a challenge especially when a woman refuses from abortion. Individual treatment approach may differ considering pregnancy terms and clinical status. Although normal childbirth is possible using IM after 2nd/3rd trimester, 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 safe DMR.

**Background:**

...chronic myeloid leukemia (CML). As increased NK cells during TKI therapy positively correlate with better outcomes, antitumor immune function of it remain to be elucidated.

**Methods:**

KIR and HLA high resolution typing were performed on peripheral blood DNA from 76 CML patients in chronic phase (CML-CP) using 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 μRNA 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 significant.

**Results:**

Second generation TKIs as first-line therapy (n=46) and female (n=29) sex were strongly associated with superior DMR at the 2-year of therapy (second generation TKIs as first-line treatment, HR 7.305, 95% CI, 3.377 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 011/000501, and 2DS4*00301 or 007/010 or 015 (HR 2.811, 95% CI, 1.590 to 4.968; p<0.001). Interestingly, KIR3DL1*00501 for the patients has more strong link with these two factors, several KIR alleles positively correlates with superior DMR at the 2-year; KIR2DL4*008 or 011/000501, 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 haploype. 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.

**E1068 IMPACT OF KIR3DL1*00501 IN TYROSINE KINASE INHIBITOR-TREATED CML**


1Division, Hematology, Tokyo Medical and Dental University, Tokyo, Japan, 2Dep.Hematology and Oncology, Kyoto University, 3HLA Foundation, Kyoto, 4Center for Comprehensive Community Medicine, Japan, 5Saga city, Japan

**Background:**

The BCR-ABL1 tyrosine kinase inhibitors (TKIs) dramatically improved long-term survival of the patients with chronic myeloid leukemia (CML). As increased NK cells during TKI therapy positively correlate with better outcomes, antitumor immune function of it remain to be elucidated.

**Methods:**

KIR and HLA high resolution typing were performed on peripheral blood DNA from 76 CML patients in chronic phase (CML-CP) using 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 μRNA 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 significant.

**Results:**

Second generation TKIs as first-line therapy (n=46) and female (n=29) sex were strongly associated with superior DMR at the 2-year of therapy (second generation TKIs as first-line treatment, HR 7.305, 95% CI, 3.377 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 011/000501, and 2DS4*00301 or 007/010 or 015 (HR 2.811, 95% CI, 1.590 to 4.968; p<0.001). Interestingly, KIR3DL1*00501 for the patients has more strong link with these two factors, several KIR alleles positively correlates with superior DMR at the 2-year; KIR2DL4*008 or 011/000501, 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 haploype. 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.

**E1069 COMPARISON OF MOLECULAR KINETICS AFTER THE FIRST AND SECOND IMATINIB DISCONTINUATION: RESULTS FROM THE KID STUDY**


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Background: Recent reports have demonstrated that tyrosine kinase inhibitors (TKIs) discontinuation can be employed in chronic phase chronic myeloid leukemia (CP CML) patients with sustained deep molecular responses after enough TKI therapy. Consequently, treatment-free remission (TFR) has been a new therapeutic goal. Although 50-70% of patients experienced molecular relapse by several imatinib (IM) discontinuation studies, the most of patients resumed molecular responses (MR) following restart of IM. Aim: We evaluated the impact of molecular response by 24 months for molecular level after 3 months (BCR-ABL1 transcript level ≤1%) of radotinib 300mg bid approved for CP CML patients who were treated with HU (1-3 g/day) because of side effects. In addition, resistance against ponatinib may develop in two study groups, early molecular response (EMR) at 3 months were evaluated. And, total of 151 patients who received 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, if MMR was not achieved, IM treatment was re-introduced. After sustaining 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, 24.8-84.5 months). After first attempt of IM discontinuation, they relapsed after a median duration of 3.7 months (range, 1.0-24.8 months) and 2 years (range, 1.0-115.1 months) after IM resumption. After sustaining a second UMRD for a median of 25.5 months, IM therapy discontinued for a second time. After a median follow-up of 8.8 months (range, 0.3-38.1 months) after second IM discontinuation, 10/12 patients (83%) and 8/12 patients (67%) lost UMRD but not MMR, one patient showed fluctuation of BCR-ABL1 transcript under the level of 0.1% on IS for 9.4 months and another patient have shown gradually increasing BCR-ABL1 transcripts under the level of 0.1%. Eight patients who experienced second relapse (MMR loss) after a median 2.9 months (range, 1.9-30.7 months). The patients who lost MMR, except one patient, were retreated with IM for a median of 7.1 months (range, 0.8-24.8 months); five patients re-achieved MMR at a median of 1.8 months (range, 1.0-10.2 months) and one re-achieved UMRD at 5.5 months.

Summary/Conclusions: With minimum 24 months follow-up, early responses at 3 months or 6 months can predict better outcomes in newly diagnosed chronic myeloid leukemia patients treated with radotinib or imatinib. But, to evaluate the significant long-term prognostic value such as overall survival and progression-free survival by EMR, longer follow-up are needed.
Results: HU treatment resulted in WBC stabilization in 3 of 4 patients, but failed to induce a molecular response. However, surprisingly, the percentage of BCR-ABL decreased significantly in all 4 patients during HU treatment and was no longer detectable in 3 of 4 cases. Stem cell transplantation could be performed in 2 patients after 2-3 months. In one patient, stable disease over 18 months was obtained with HU-therapy. In one patient, the disease progressed rapidly despite temporary suppression of BCR-ABL. In vitro studies, HU was found to block the growth in all cell lines tested and in all primary cell samples (n=7) examined, with IC50 values ranging between 50 and 250 µM. Interestingly, cell lines exhibiting mutant BCR-ABL1 were more sensitive against HU than cell lines expressing BCR-ABL1 WT. HU and ponatinib were found to synergize in inhibiting growth of all cell lines tested, including cells expressing BCR-ABL1 T315I or T315I including compound mutations. Cooperative drug effects were also confirmed in primary CML cells (n=4). In cell line experiments, ponatinib was found to suppress Ba/F3/T315I cells but not Ba/F3/T315I/F359V or Ba/F3/T315I/E255V cells, whereas HU was found to exert stronger effects on cells expressing mutant BCR-ABL1, and the drug combination resulted in complete suppression of all sub-clones. Summary/Conclusions: Our data show that HU exerts strong, sub-clone specific, specific, anti-neoplastic effects in TKI-resistant CML cells. Clinical studies are now warranted to define the exact value of the drug combination ponatinib+HU in TKI resistant CML.

E1072
ASSOCIATION OF BCL2L11 (BIM) DELETION POLYMORPHISM WITH MOLECULAR RELAPSE AFTER TYROSEINE KINASE INHIBITOR CESSION IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH DEEP MOLECULAR RESPONSE
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Background: The inhibition of BCR-ABL kinase with tyrosine kinase inhibitors (TKIs) has markedly improved the prognosis of chronic myeloid leukemia (CML). Recently, it has been recognized that some CML patients with deep molecular response (DMR) can maintain treatment-free remission (TFR) after TKI cessation. Recently, it has been recognized that some CML patients with deep molecular response (DMR) can maintain treatment-free remission (TFR) after TKI cessation. To further clarify the role of predictive biomarkers in molecular relapse after TKI cessation, we performed a long-term follow-up of CML patients with DMR after TKI cessation.Methods: Patients with DMR receiving TKI treatment were included. Molecular relapse was defined as a loss of the major molecular response (MMR). The molecular response was considered as a loss of the major molecular response (MMR). The present studies were designed to verify the limit of detection (LoD) for Xpert® BCR-ABL Ultra assay 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 Xpert® BCR-ABL Ultra assay 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.

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 cell lines with initial BCR-ABL1 levels >10% (IS) and pooled blood from CML negative patients, ranged from 10% to <0.001% (IS). Twenty-one replicates of each dilution 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% (IS) (SD=0.17 Log) to 0.0011% (IS) (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 MR4.5 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 INCIDENT CASES OF GAUCHER DISEASE IN SPLENOMEALGY AND/OR THROMBOCYTOPENIA PATIENTS IN SPECIALIZED MEDICAL SERVICES IN COLOMBIA THROUGH THE USE OF A SELECTION ALGORITHM


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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 reported 86% cases with moderate to severe splenomegaly and 60% thrombocytopenia at the time of diagnosis, thus demonstrating why patients are referred to hematology. A diagnosis of GD is considered after other diagnostic hypotheses have been ruled out. The consensus of international experts on the management of patients with GD established a diagnostic algorithm that is particularly intended for specialists (Mistry, 2010). Straightforward implementation of diagnostic algorithms to support medical specialties in Latin America for early diagnostic testing of GD is required.

Aims: To identify new cases of GD in a selected population with splenomegaly and/or thrombocytopenia referred to Hematology, Pediatric Hematology, Pediatrics and Internal Medicine, using a selection algorithm for the genotyping of GD patients.

Methods: Multicenter, descriptive study, in active recruitment process with non-probabilistic sampling by convenience. Currently, the study has 51 specialized medical centers in Hematology, Pediatrics and Internal Medicine in Colombia, approved by Ethics Committee (EC). The study has an expected duration of 24 months since EC approval for each center. Eligible subjects are those with three documented criteria: thrombocytopenia (<150,000/cc plus anemia (hemoglobin <110g/dl in men and <11 g/dl in women) plus/or bone pain plus/or monocular Gammopathy of Unknown Significance plus/or Polyclonal Gammopathy in subjects aged 30 years and older; and/or splenomegaly defined as palpable spleen ≤1cm below the costal rib or diagnosed by imaging, and/or Splenectomy by splenomegaly with no known cause. Subjects with prior diagnosis of GD, splenomegaly due to portal hypertension, hematologic malignancy, hemolytic anemia and thalassemia were excluded. Informed consent was obtained for all included subjects. Clinical information was collected from their medical history. The enzymatic activity of the β-glucocerebrosidase was performed in peripheral blood, using dried blood spots (DBS) and/or leukocytes. In subjects with reduced enzymatic activity in DBS, confirmed β-glucocerebrosidase deficiency and symptomatic GD, a bone marrow biopsy was performed to diagnose the cases of GD.

Results: Since Feb/14 to Nov/16, 400 subjects have been included (51.3% men) with a median age of 28.79 years (range, 0.01 to 91.87). Reduced enzymatic activity of β-glucocerebrosidase was identified in 14 subjects (50% men) with a median age of 12.68 years (range, 0.3 to 74.85). All subjects were non-Ashkenazi origin, with 82.8% thrombocytopenia, 49.5% splenomegaly and 4.33% splenectomy. Detailed population description is on Figure 1.

Summary/Conclusions: This study suggests that selection algorithm could be implemented in Colombia, supporting specialists in making decisions on diagnosis of Gaucher Disease. Further characterization of the population is ongoing.

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E1075
IMPACT OF PEROXIREDOXIN 2, GLUTATHIONE Peroxidase and Catalase Inhibition on Oxidative Stress Modifications of Red Blood Cell Membrane and Cytosol

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Background: Several anemias are associated with oxidative stress, namely, sickle cell anemia, β-thalassemia, glucose-6-phosphate dehydrogenase deficiency and hereditary spherocytosis. Red blood cells (RBC) are continuously exposed to reactive oxygen species (ROS), triggering oxidative modifications. The antioxidant system, however, when its capacity is overwhelmed, the cell is exposed to oxidative modifications of the RBC, as showed 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 simultaneously, leads to oxidative stress modifications within the RBC, as showed 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 antioxidant defense.

Aims: We aimed to study the importance of Prx2, GPx and CAT inhibition on defense against oxidative stress in normal erythrocytes.

Methods: We performed in vitro assays (n=3) with RBCs from healthy volunteers, inhibiting Prx2, GPx and CAT, either individually, two-by-two or all three; concomitant inhibition 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, cytosols’s total antioxidant status (TAS), by spectrophotometric methods.

Results: Concerning TAS we found a trend towards decreasing values with enzyme inhibition (one or more); the lowest value of TAS was observed when all three enzymes were inhibited and, when only two enzymes were inhibited, the lower values were obtained for pairs that included CAT inhibition; when only one enzyme was inhibited, GPx inhibition showed the highest TAS; regarding LPO, a trend towards increasing values with enzyme inhibition was observed; the lowest value was obtained when all enzymes were active, and the highest when all of them were inhibited; when only one enzyme was inhibited, CAT inhibition showed the highest LPO value and when two enzymes were inhibited, LPO was increased for the pairs that included GPx, MBH was increased for all enzyme inhibitory conditions, when compared to the condition with all enzymes active, excepting when CAT was inhibited.

Summary/Conclusions: In conclusion, inhibition of these antioxidant enzymes, either alone or simultaneously, leads to oxidative stress modifications within the RBC, as showed 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 antioxidant defense.

Figure 1.
antioxidant homeostasis, and suggests that inhibition or injury to one (or more) compromises erythrocytes, which might influence clinical presentation in oxidative stress associated anemias.

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E1076

MOLECULAR BASIS OF PKLR MUTATIONS IN PATIENTS WITH PYRUVATE KINASE (PK) DEFICIENCY: THE FIRST REPORT FROM SOUTHEAST ASIAN POPULATION

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Background: Recently we have identified a new form of transfusion dependent hemolytic anemia due to KLF1 mutations causing a trans-acting deactivation of pyruvate kinase genes (PKLR). Mutations of PKLR per se can affect red blood cells metabolism and cause a wide range of clinical manifestation from fetal anemia leading to hydropic fetus, severe neonatal jaundice requiring multiple exchange blood transfusions, chronic to fully compensated hemolytic anemia. Understanding of the molecular basis of pyruvate kinase deficiency (PK def.) might be useful to predict clinical phenotypes and suggest appropriate clinical management of future patients. Moreover, an interaction of PKLR and KLF1 mutations in such patient has not been explored.

Aims: This study aim to identify the mutation of patients with PK def. for the first time in Southeast Asian populations.

Methods: Seven unrelated patients; 6 from Thailand and 1 from Indonesia have been enrolled after inform 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 become 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 11 different mutations in 5 compound heterozygotes and 1 homozygote as shown in Table 1. Four mutations appeared to be novel as they were not been reported in any public databases (c.1269+3A>G; c.353A>G =p.N118S; c.941T>C), mismatched PCR-RFLP for c.1403C>G, c.1463G>A and IVS9(+3)A>G). Interestingly, one index 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 splenomegaly, peripheral blood cytopenias (mostly thrombocytopenia and/or anemia), growth retardation, bone involvement, gammapathies, increased risk of malignancies and, in some patients, neurological manifestations. Since symptoms are non-specific, the diagnosis can be delayed for years or missed. Enzyme replacement therapy (ERT) with recombinant β-glucocerebrosidase is safe and effective in preventing and/or reversing many clinical manifestations. However, if the diagnosis is delayed for years, major complications cannot be reversed. A useful screening method for GD is based on measuring enzyme activity on a Dried Blood Spot (DBS), while the gold standard test is still considered GBA activity in cellular homogenates. A pediatric algorithm has been proposed to promote timely diagnosis and early access to ERT (figure 1).
evaluate the prevalence of GD among children referred to the haematology paediatric units and selected according to the above mentioned diagnostic algorithm. Here, we report a preliminary analysis of GAU-PED trial.

Methods: The GAU-PED study involves 53 centers in the context of the AIEOP Study Group, the Italian clinical research consortium in paediatric haematology and oncology. Patients referring to the pediatric hematology and oncology units for evaluation of a hematological condition with hemolysis (hemolytic anemia and/or anaemia), where other causes of splenomegaly has been excluded, are tested for G6PD activity though a DBS sample. Only patients with DBS showing a G6PD activity below normal values are recalled to confirm G6PD enzyme deficiency using the gold standard G6PD analysis in cell homogenate. For every tested patient, clinical information are also collected.

Results: After parental consent, a total of 25 DBS have been collected from 11 centers, in the first 12 months of study accrual. DBS values under 4.4 pmol/punch –7/nmol/ were found in 9/25 patients (36%). These DBS have been recalled for the conventional enzymatic test. The diagnosis of GD has been confirmed in 5/9 patients (55%), with a prevalence of GD of 20% (95% CI: 8.3-39.1%) in 20% (95% CI: 8.3-39.1%) of patients aged ≥60 years, p<0.001, 70-79 years, p<0.001, ≥80 years, p<0.001). Anemia was most frequently encountered among men aged ≥80 years, p<0.001, 70-79 years, p<0.001. Anemia was present in 10.3% of patients aged 60-69, in 20.1% of those aged 70-79 and in 36.5% 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). Anemia was mild in 69.8% of patients, but a severe form was significantly more often among men aged ≥80 years (p<0.03). Analysis of the etiology of anemia revealed three predominant types: anemia of chronic disease (33.1%), unexplained anemia (28.4%) and deficiency anemia (22.5%), including iron deficiency 13%. In patients with anemia, those with anemia were older (p<0.001), had a higher prevalence of comorbidities (p<0.001) and were more often hospitalized (p<0.001). In the multivariate logistic regression model, factors increasing the risk of anemia were: age (≥80 years OR=2.29; 95%CI 1.19-4.42; p=0.013), 70-79 years OR=2.85; 95%CI 1.12-7.3; =0.029, 3 diseases OR=6.28; 95%CI 2.22-17.76; p=0.001, 4 diseases OR=6.4; 95%CI 1.27-17.01; p=0.021) and the number of hospitalizations (OR=1.34; 95%CI 1.13-1.58; p=0.001). At the end of the 2-yr follow-up, the cumulative survival among patients without anemia in relation to the group with anemia was 90.76% vs 78.08% and the difference was statistically significant (p<0.001). In multivariate model, factors that significantly increased the risk of death in study population were anemia (HR=3.33; 95%CI 1.43-7.74; p=0.005), cancer (HR=3.31; 95%CI 1.47-7.49; p=0.004) and heart failure (HR=2.94; 95%CI 1.33-6.51; p=0.008).

Summary/Conclusions: In patients ≥60 years the incidence of anemia increases with age, gender and male predominance. Comorbidities and frequency of hospitalization. The high rate of unexplained anemia indicates the necessity for detailed hematologic diagnosis. The occurrence of anemia among people aged ≥60 years has an adverse impact on survival.

Table 1.

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.

E1078

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, mRNA 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, haemolytic uremic syndrome, and thrombotic thrombocytopenic purpura.

Aims: To evaluate platelet MPs (PMP), tissue factor expressing MPs (TFMs), endothelial MPs (EMPs) and microparticles expressing single antigens (CD41, CD142 and CD144) levels in in other haemolytic anaemias, such as hereditary spherocytosis (HS), elliptocytosis (HE), stomatocytosis (HSI), red cell enzymatic defects, congenital dyserythropoietic anaemia (CDA), autoimmune haemolitic 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 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 V Binding buffer and 25 µl of Fixed Fluor reagent was 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 months (range 2-34) and compared with normal controls. The median number of MPs in PFP was 2.85 x 104 (range 22-87) in controls, 9/43 (21%) had been splenectomized and 13/43 (30%) were treated at the moment of the study (steroids/immunosuppressors for AIHA, and eltuzumab for PNH). Table shows Hb levels, PLT and WBC counts of the different patient groups. In AIHA, the median age of patients (15 male and 28 female) was 53 years (range 22-87), while the mean time between the DBS test and the diagnosis has been 2 months. ERT has been started in all GD patients.

Summary/Conclusions: Our preliminary results support the use of DBS as screening test for GD in a selected population of children with splenomegaly and/or thrombocytopenia considered at increased risk for the disease. The use of a 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.
E1080
PIEZO1 MECHANOTRANSDUCTIVE PROTEIN MUTATIONS IN RBCS: WHEN THE PHENOTYPE IS BEYOND HYALOMYCIST ANAEMIA
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Background: Piezo proteins are integral membrane proteins with many transmembrane domains broadly expressed, including erythrocytes (RBCs). PIEZO1 proteins play an important role as an osmoreceptor, maintaining RBCs ionic homeostasis, functioning as a mechanically activated cation channels. Mutated PIEZO1 proteins have been linked to hereditary xerocytosis (HX), which is characterized by RBCs dehydration with mild to moderate compensated haemolytic anaemia and iron overload. As these clinical features are present in many different clinical conditions, the diagnosis always needs a high level of suspicion. Nowadays, besides peripheral blood smear (PBS) observation, molecular analysis, searching for mutations in PIEZO1 gene, became a tool in the diagnosis of HX.

Aims: Describe 26 patients with HX associated with PIEZO1 mutations belonging to 13 unrelated families, raising awareness of the highly variable phenotype of this patient, and the need of a high grade of suspicion along with the morphologic evaluation of the PBS.

Methods: Collection of clinical and laboratory data on our 26 patients with HX and hyperferremia due to 10 different identified mutations in PIEZO1. Sanger sequencing was used to identify mutations affecting PIEZO1, encoded by FAM38A gene, and to confirm transmission according to the presence of disease phenotype. In all patients were excluded other known causes of hyperferremia (HF) and haemolytic anaemia.

Results: Of the 26 patients identified as having PIEZO1 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 reticulocyte counts of a median reticulocyte count of 101 x 10^9/L (28.1-557.3), 18/26 patients had HF with a mean value of ferritin of 556 ng/mL (161-6817) and 9/26 had both. Of the 26 patients, four had splenomegaly and six gallbladder lysis (5/6 cholecystectomized), two of them both. Only 5 patients presented with anaemia (Hb <12 g/dL), 2 macrocytic and 3 normocytic. One patient had coexisting sickle cell disease and he also had a xerocytosis 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 pathology has multiorgan involvement that is related to the degree of hemolysis. This iron overload may be related to a defective iron homeostasis dependent on PIEZO1 function not strictly related with Xerocytosis.

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 electrophoresis and ESA assay. PIEZO1 and KCN4 coding regions were analyzed by Sanger sequencing in all HX patients. Hemoglobin oxygen affinity was evaluated using p50 measured on venous blood on a Hemoxanalyser 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 KCN4. Five families (9 subjects) have already been reported, with identified mutations in exons 18, 21, 42 or 51. Five subjects from 4 new families carried new mutations in exons 14, 16 and 20 for which bioinformatic softwares showed a high likelihood of pathogenicity. For all HX patients, p50 values were under the normal range (mean 21.1, range 19.7-23.4, normal range 25-29 mmHg), contrasting with HS 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 oxygen affinity we observed. In particular, we found a radiocytosis 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.

E1081
MODELLING PYRUVATE KINASE DEFICIENCY IN HUMAN PROGENITORS USING CRISPR/CAS9
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Background: Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene. PKD produces chronic non-spherocytic hemolytic anemia, which can be fatal during early childhood and may require transfusion dependence. 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 (coRPK). 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: DRRU-2016-5168).

Aims: To test the efficacy of the therapeutic LV, we have proposed an alternative to the patient-derived 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.

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Gene therapy, cellular immunotherapy and vaccination

**E1083 SAFETY AND EFFICACY OF MULTI-PATHOGEN-SPECIFIC T CELLS IN A HUMANIZED MODEL OF INVASIVE ASPERGILLOSIS: A PROOF OF CONCEPT STUDY**

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Background: Viral infections, most commonly by cytomegalovirus (CMV), Epstein-Barr virus (EBV), polyoma virus type 1 (BK), and fungal infections, main-
ly by Aspergillus Fumigatus (Asp), are leading causes of transplant-associated mortality in patients undergoing allogeneic hematopoietic stem cell transplan-
tation. 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 attrac-
tive alternative. Towards unleashing its full potential and treat multiple viral and fungal infections by a single T-cell product, we developed a rapid, simplified and minimally laborious protocol for the generation of multipathogen-specific T cells (mp-STs) that simultaneously target CMV, EBV, 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 1.5x10^7 of immunomagnetically isolated CD3+ cells (donor lymphocyte infu-
sion) were retransferred into lethally irradiated nude mice intranasally inoculated with Asp conidia or left uninfected. Mice were monitored for 28 days. Results: mp-STs succumbed early, before aGvHD development. Non-specific DLI failed to control IA despite T-
specific against Asp [spot forming cells (SFC)/2x10^5 cells: 315±82] and the targeted
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Background: Cytotoxic T-cell (CTL) responses against malignant cells play a major role in maintaining remission and prolonging overall survival in patients with hematologic malignancies after allogeneic stem cell transplantation (allo-
SCT) and/or donor lymphocyte infusions (DLI). Graft versus leukemia (GvL) effects after allogeneic stem cell transplantation and/or DLI are considered to be T cell-mediated. Many groups described specific T-cell responses against several leukemia-associated antigens (LAA) in different hematological malig-
nancies. However, T cell responses after allo-SCT and DLI are not well char-
acterized.

Aims: In this study, we analyzed LAA-specific T cell responses after allo-SCT and DLI. To this end, we assessed the frequency and diversity of LAA-specific T cell responses using ELISPOT analysis and tetramer assay in 12 patients (5 patients (pts) with acute myeloid leukemia, 2 pts with chronic myeloid leukemia, 3 pts with multiple myeloma and 2 pts with chronic lymphatic leukemia) before and after DLI. Epitopes derived from PRAME, NPM1/MLH, RHHAMM, WT-1 and other LAA were tested. Moreover, the frequency of regulatory T (Treg) cells was measured and the course of cytokine profiles before and after DLI was analyzed. These immunological findings were correlated to the clinical course in the respective patients.

Methods: In ELISPOT and tetramer assays, an increase in frequency and diver-
sity of LAA-specific T cells was observed in all patients. Cytokine assays using ELISA for the detection of more than 10 cytokines before and after DLI were employed.

Results: Importantly, there was a significant increase from 0 to 7 LAA-derived T cell epitopes (P=0.03) in clinical responders (R) when compared to non-
responders (NR). These positive results in R versus NR where confirmed by
tetramer-based flow cytometry assays, where an increase in frequency from
0.5 to 2.3% in the R group of LAA-specific T cell/all CD8+ T cells was observed.
Interestingly, the frequency of Tregs in clinical responders decreased signifi-
cantly from a median 72.9% to 54.6% (P=0.008) while the frequency of Tregs stayed stable over time in non-responding patients. T cell subset analysis did not reveal significant differences before versus after DLI administration. In cytokine assays using ELISA we found a significant increase of IL-4 after DLI.

Summary/Conclusions: Taken together, we detected an increase of specific CTL responses against several LAA after allogeneic stem cell transplantation and donor lymphocyte infusion. Moreover, this study suggests that broader LAA epitope-specific T cell responses as well as decreasing numbers of Tregs contribute to clinical outcome of patients treated with DLI.

**E1085 GENE-MODIFIED NK-92MI CELLS EXPRESSING A CHIMERIC CD16/CD64-
BB-Z RECEPTOR EXHIBIT ENHANCED CANCER-KILLING ABILITY IN COMBINATION WITH THERAPEUTIC ANTIBODY**

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Background: Natural killer (NK) cells play a pivotal role in monoclonal anti-
body-mediated immunotherapy through an antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. NK-92MI is an interleukin-2 (IL-2)-independent cell line, which was derived from NK-92 cells with superior cytotoxicity to a wide range of tumor cells in vitro and in vivo. However, the Fc-receptor (CD16), which usually mediates ADCC, is absent in NK-92 and NK-92MI cells.

Methods: Donor lymphocyte infusion in patients with hematological malignancies leads to diversity of leukemia-associated-antigen-specific T cell responses and to reduction in regulatory T cell frequency

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Figure 1. NK-92MIhCD16 and NK-92MIhCD64 functional validation in vitro and characterization. A. Schematic representation of the CD16-BB-ζ and the CD64-BB-ζ receptor constructs. B. Exogenous CD16 or CD64 expression on surfaces of NK-92MI cells are shown. C. Immunoblot analysis of CD3ζ fusion protein expression in NK-92MIhCD16 or NK-92MIhCD64 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 CD8α extracellular domain, CD28 transmembrane domains, two costimulatory domains (CD28 and 4-1BB), and the signaling domain from FcRγ. The resulting chimeric receptors, termed CD16-BB-ζ and CD64-BB-ζ, were utilized to generate chimeric receptor-modified NK-92MI cells, which were named NK-92MIhCD16 and NK-92MIhCD64 cells, respectively.

Results: We found that NK-92MIhCD16 and NK-92MIhCD64 cells significantly improved cytotoxicity against CD20-positive non-Hodgkin’s lymphoma (NHL) cells in the presence of rituximab.

Summary/Conclusions: These results suggest that the chimeric receptor-modified NK-92MI cells could potentially enhance the clinical responses mediated by currently available anticancer monoclonal antibodies (mAbs).

E1086
A NOVEL IN VITRO METHOD TO QUANTIFY THE PHARMACOLOGY ACTIVITY OF BSICIFIC ANTIBODIES IN HEMATOLOGICAL SAMPLES


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Background: The PharmaFlow automated flow platform has achieved 85% clinical correlation with AML samples with its novel Native Environment assay. Recently, novel Bi-specific antibodies (BsAbs) or analogous constructions active antitumor immunotherapy with BsAbs. The integration of Effective E:T ratio and pharmacological parameters better predict the in vitro 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 better 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 Biolabs) and 7 CLL and 3 B-ALL samples with Blinatumumab (Amgen). When appropriate, basal quantification of TAA was performed by flow cytometry (FCM). The PharmaFlow platform by FCM and the B-ALL dataset 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. Differences in T-cell lysis 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 BsAb concentrations or with a remarkable expansion of activated T-cells that suggest the use of immune checkpoint to unblock this immunoresistant status.

Summary/Conclusions: We have developed an automated flow cytometry assay for bi-specific antibodies screening that keep basal conditions (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. Differences in T-cell lysis 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 BsAb concentrations or with a remarkable expansion of activated T-cells that suggest the use of immune checkpoint to unblock this immunoresistant status.

E1087
HUMANIZED CD7 NANOBODY-BASED IMMUNOTOXINS EXHIBIT PROMISING ANTI-T-CELL ACUTE LYMPHОBLASTIC LEUKEMIA POTENTIAL

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Background: Nanobodies, or named as VHs, are derived from heavy-chain-only antibodies that circulate in sera of camels. Their exceptional physico-chemical properties, possibility of humanization and unique antigen recognition properties make them excellent candidates for targeting delivery of biologically active components. In our previous work, we have successfully generated the monovalent and bivalent CD7 nanobody-based immunotoxins, which can effectively trigger the apoptosis of CD7 positive malignant cells.

Aims: To pursue the possibility of translating these immunotoxins into clinics, we humanized the nanobody sequences (designated as dhuVH6H6), 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, dhuVH6H6-PE38, dVH6-PE-LR, and dhuVH6H6-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 the ability to bind specifically to CD7-positive T lymphocyte with a strong signal corre-
Summary/Conclusions: We have successfully constructed a targeted CD7 molecule modified nanobody (CD7 molecule improved nanobody) immunotoxin dhuVH66-PE38 and showed its potential for treating CD7-positive malignant tumors, especially T-cell acute lymphoblastic leukemia.

E1088
STATINS MAY IMPROVE CAR-NK IMMUNOTHERAPY IN MM BY PREVENTING LOSS OF BCMA EXPRESSION ON MM CELLS

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Background: Chimeric Antigen Receptor (CAR) modified immune cells targeting BCMA against multiple myeloma (MM) has appeared as a feasible immunotherapy strategy to treat MM patients. However, high doses of CAR immune cells are required to achieve a response. Cord blood derived NK cells (CB-NK) is a feasible source of obtain NK cells to modify with a CAR against BCMA. We previously observed that MM cells exposed to CB-NK are able to transfer MM proteins, such as BCMA, both to CB-NK and to adjacent MM cells non-exposed to CB-NK. Furthermore, statins, which are toxic for MM cells, by altering the lipid composition of tumor cell membrane are involved in cell-cell communication. We hypothesized that statins could prevent the loss of BCMA exposed by the loss of BCMA sum cells after CB-NK exposure, allowing infusing a lower CAR immune cell dose in MM patients.

Aims: To evaluate the effect of statins on MM cell proliferation, on the CB-NK immune response against MM, and on BCMA expression in MM cells after CB-NK exposure.

Methods: The cytotoxicity of statins against MM cells was determined in vitro and in vivo in a murine MM model; furthermore, their impact in CB-NK cytotoxicity against MM was also determined in vitro. BCMA expression on MM cells after CB-NK exposure was analyzed by confocal microscopy and by flow cytometry. FACS sorting experiments were performed to analyze BCMA transfer between CB-NK exposed MM cells to neighboring non-exposed CB-NK MM cells.

Results: Atorvastatin and Fluvastatin treatment (1µM) decreased MM cell line (ARP1, RPMI, KMM1) proliferation. No effect was detected for U266 MM cells and for K562 non-MM cells. In vivo studies, showed that mice treated for three days I.P with Fluvastatin (1mg/kg) showed significant decreased MM disease progression. Blocking of BCMA decreased CB-NK cytotoxicity against MM cells. Furthermore, pretreatment of MM cells with Fluvastatin (3 µM) increased CB-NK cytotoxicity against all MM cell lines; no impact was observed against K562 non-MM cells. Co-culture experiments showed that, as soon as 30 minutes, CB-NK exposure led to a BCMA transfer from MM cells to CB-NK and to the extra-cellular milieu leading to a loss of BCMA expression on MM cells. Fluvastatin pretreatment prevented loss of BCMA expression. After two days of co-culture, alive MM cells still showed decreased BCMA surface expression, and surprisingly, increased intracellular BCMA expression. Fluvastatin pretreatment partially avoided and alive MM cells still showed decreased BCMA surface expression, and surprisingly, increased intracellular BCMA expression. 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 CB-NK 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 an emerging evidence that the maximal benefit of dendritic cell (DC)-based cancer immunotherapy may be achieved by combination with other therapies that act to immunomodulation and tumor microenvironment.

Aims: In this study, we tried to obtain the best efficacy of immunotherapy using DC vaccination in combination with lenalidomide and PD-1 blockade in a murine myeloma model.

Methods: After establishing myeloma-bearing mice, four treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) DCs + lenalidomide, 4) DCs + PD-1 blockade, and 5) DCs + lenalidomide + PD-1 blockade. After treatment, preclinical response and in 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 immunom-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.

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 amp. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating differences due to somatic mutation.

Aims: Our goal was to develop an NGS assay based on AMP that would enable IR characterization utilizing a minimal set of unidirectional GSPs and to reduce amplification bias through the use of MBC adapters.

Methods: Upon developing our AMP-based NGS assay, we validated its quantitatively reproducible sensitivity 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, Immunovereux™ (IGH, TRB, and TRA) for B-cell and T-cell repertoire sequencing, respectively. Both assays demonstrated high reproducibility between replicates with quantitative clone tracking down to 0.01%. The ability to determine clonotype and clonotype IGVH 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 mimetic 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 DEXA strongly inhibited tumor growth, compared with other groups. in vitro immunological analyses revealed that these enhanced anti-tumor effects were closely associated with the decrease of regulatory cell populations, such as regulatory T cells (Treg) and type 2 macrophages (M2), and the increase of effector cell populations, including activated CD4 T cells, and type 1 macrophages (M1), accompanied with the activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells in the splenocytes from vaccinated mice.

Summary/Conclusions: This study suggested that the DC combined with Pom and DEXA synergistically enhanced the anti-tumor immunity in a murine myeloma model, by skewing immunosuppressive status toward immunosuppressive status in tumor microenvironment.
ALTERNATIONS 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-culture with some MSCs samples. On lymphocytes derived from one donor the elevation of HLA-DR was observed after co-cultivation with half of MSCs samples (group A), on the others the HLA-DR expression level did not change (group B). MSCs were divided into two groups based on HLA-DR rise on lymphocytes. Study of T-cell subpopulations after interactions with MSCs could explain ineffectiveness of some MSCs as an immunomodulating agent in clinical applications.

Aims: The aim of the study was to discriminate variations in T-cell subpopulations, co-cultured with MSCs from two groups.

Methods: MSCs were isolated from bone marrow of 13 donors for allogeneic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10⁵ cells per flask, and then 10⁶ allogeneic lymphocytes from single donor were added to all MSCs cultures. For lymphocytes activation 5μg/ml phytohaemagglutinin (PHA) was added to half of these cultures. Lymphocytes were removed from MSCs. Then 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 expressions were compared. Subpopulations which significantly differed between groups A and B were compared. The results were presented as median ± SEM.

Table 1.

Results: Expression of HLA-DR on lymphocytes after 4 days of cultivation without MSCs did not change compared to 1st day. When lymphocytes were co-cultured with some MSCs samples expression of HLA-DR was higher. Elevated percentage of HLA-DR positive cells correlates between CD4+ and CD8+ cells (R²=0.932). Thus samples of MSCs were divided into two groups: in group A proportion of HLA-DR lymphocytes was 3 times greater than in group B. Subpopulations of lymphocytes co-cultured with MSCs from group A and B were compared. Subpopulations which significantly differed between groups A and B are presented in the table. In lymphocytes co-cultured with MSCs there were higher number of naïve cells compared to control (47.4±3.5% and 54.9±2.0% for group A and B 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 (MF) in group A than in group B (635±130 vs 289±18, p=0.03). These data indicated that MSCs from group A had become more immunogenic after interaction with lymphocytes and could not show immunomodulating properties in same way as MSCs from group B.

Summary/Conclusions: The immunomodulatory properties of MSCs depend on the donor. This could explain why administration of MSCs is not always successful. Preliminary study of MSCs prior to their administration may be used to predict their efficiency in the future.

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Results: Although a high toxicity and low efficiency were observed with the electroporation technique used, up to 96% colony forming units showed the specific integration. Experiments directed to improve efficacy and reduce toxicity were then conducted. A high percentage of gene edited HPCs were detected by shortening the cell expansion and puromycin selection periods. Importantly, gene edited HPCs were detected after infusion in immunodeficient (NSG) mice. More recently, CRISPR-Cas9 genome engineering 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.

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 without side effects. 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, HLADR and PD-1 were studied by flow cytometry as well as distribution of naïve and effector memory cells in lymphocytes and MSCs. The proportion of these cells was analyzed over time. Lymphocytes and MSCs were analyzed using flow cytometry.

Results: By the fourth day of incubation the proportion of naïve CD4+ cells reduced from 30% (from 47.5±3.0% to 32.8±3.3%) in cultured lymphocytes. It did not happen in lymphocytes co-cultured with MSCs and gMSCs (p=0.001). At the same time in cultured lymphocytes the fourth day of the number of CD4+ central 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 transition into effector cells. The proportion of CD4+/PD-1+ cells increased from 8.8±1.1% to 10.9±0.7% by the 4th day of cultivation. When co-cultured with MSCs and gMSCs the proportion of PD-1+ cells decreased from 14.3±2.7% to 12.5±1.5% (p=0.0125).

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 CD4+ central memory cells increased from 24.4±2.7% to 46±4.5% (p<0.047). Thus the interaction of PHA-lymphocytes with MSCs inhibited their activation and preserved naïve state.

Summary/Conclusions: The composition of lymphocyte population changes during cultivation. The proportion of naïve cells reduced, while the number of effector cells and the proportion of PD-1+ increased, indicating the lymphocyte activation probably due to the presence of xenogenic serum in the culture medium. Co-cultivation with MSCs maintained lymphocytes in not activated state. The interaction of activated lymphocytes with MSCs inhibits their activation and preserves naïve state. IFN-γ priming did not enhance MSCs inhibitory effect. The lymphocyte activation shown that MSCs both in naïve lymphocyte condition and have an inhibitory effect on their activation.

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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 transplantation 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-dependent despite splenectomy, which confers increased risks of infections. Preclinical gene therapy studies conducted in pyruvate kinase deficient mice have shown the safety and the efficacy of a new PCCorPKW-17 therapeutic lentiviral vector that has been granted orphan drug designation (EMA: EU/3/14/1330; FDA: DRU-2016-5168).

Summary/Conclusions: The use of G-CSF primed halo-identical microtransplantation appears to be a biologically active therapy in patients with refractory AML, especially in patients received less than four previous chemotherapy, fludarabine-free previous chemotherapy, response naïve and young age patients.

E1098 BLAST KINETICS AFTER NON-ENGRAFTMENT HAPLOIDENTICAL MICROTRANSPLANTATION IN PATIENTS WITH REFRACtORY ACUTE MYELOID LEUKEMIA

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Background: Multiple trials have showed that granulocyte colony-stimulating factor (G-CSF)- mobilized donor peripheral-blood stem cells (GPBSCs) based on allo-graft can be effective in mediating graft-versus-leukemia (GVL) effects and promote hematologic recovery without triggering of acute GVHD.

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 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.

Figure 1.

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 attained neutrophil recovery with median time 29 days (range, 13-40 days), while the other four patient did not. Two patients attain platelet recovery with median time 34.5 days (range, 29-40 days), while the other five patient did not. The cellular therapy did not elicit statistically significant changes in bone marrow blast % over time, F(2, 10)=1.558, p=0.258, partial n2=0.03, with bone marrow blast % decreasing from pre-infusion blast % (M=60%, SD=22.4%) to D14 blast % (M=39.5%, SD=24.47%) then increased to D30 blast % (M=54.8, SD=33.5%), in that order. Less than four previous chemotherapy, fludarabine-free previous chemotherapy and response naïve patients are the factors associated with good response to microtransplantation.

There was a strong positive correlation between patient age (statistically significant), CR1 duration (statistically non-significant) and blast % at D30, r= 0.24 and .693, p=0.036 and p=0.307 respectively. There was a moderate negative non-statistically significant correlation between CD34+ cell dose and blast % at D30, r=-0.315.
E1098

INTERACTION OF MULTIPOTENT MESENCHYMAL STROMAL CELLS WITH LYMPHOCYTES REDUCES THEIR IMMUNO PRIVILEGED PROPERTY

With IFNγ treatment of MSCs, the immunophenotypic and functional properties of MSCs are changed, leading to a decrease in their immunopriviledge property and an increase in their immunomodulatory properties. IFNγ treatment increases the expression of activating ligands on MSCs, such as ICAM1 and HLA-DR, which enhances their interaction with lymphocytes.

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 (VCN) by qPCR ranged from 0.5 to 3 VCN/ncell, 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, increasing the VCN up to 2-fold.

Summary/Conclusions:

Transduction optimizations are being carried out in order to reduce the amount of viral vector needed to achieve optimal transduction efficiencies.

E1100

C-TYPE LECTIN-LIKE RECEPTOR 2 SPECIFIES A FUNCTIONALY 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 immunophenotytes 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). This subset, which we refer to as CLEC2+HSCs, displays a unique combination of immunophenotypes and functional properties.

Methods:

To define the functional subpopulations of early hematopoietic progenitors, we performed a comprehensive analysis of CLEC2+HSCs in the context of their hematopoietic differentiation and interaction with other cell types.

Results:

CLEC2+HSCs exhibit unique immunophenotypic characteristics and a distinct differentiation potential, suggesting that they represent a distinct functional subset of HSCs.

Summary/Conclusions:

We have defined the window of mammalian HSC specification. The abrupt loss of ongoing HSC specification at E10.25 suggests an active mechanism that terminates this process. We also observed large phenotypic and functional variability amongst individual HSC precursors examined throughout ontogeny.
Aims: In this study, we analyzed in vivo dynamics of CLEC2^high^ 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 M^{K}P, respectively. We performed transplantation assays using HSCs isolated from EGFP transgenic (CAG-E{sup EGFP}) mice to trace donor-derived HSCs and their progeny by excising enucleated +/−{sup EGFP} recipients and CLEC2^high^ and CLEC2^low^ 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 1x{sup 10^7} BM cells from the recipient mice 16 weeks after the 1st transplantation.

Results: Bone marrow analysis revealed that both EGFP^+^CLEC2^high^ and CLEC2^low^ donor-derived HSC populations were detected for up to 12 weeks after transplantation. Also, these subsets were capable of generating all lineages of cells in transplanted mice. Interestingly, CLEC2^high^ HSCs generated CLEC2^high^ progeny more efficiently than EGFP^+^ HSCs in short-term grafts (1 to 2 weeks after transplantation) (p<0.05). Consistent with these reciprocal transition, both types of HSCs could effectively reconstituted hematopoiesis in the secondary recipients. However, CLEC2^high^ HSCs showed significantly reduced repopulating activity than CLEC2^low^ cells, especially at 12 weeks after transplantation (mean of HSC proportion in the primary recipients with CLEC2^high^ HSCs vs CLEC2^low^ HSCs (each n=5): 21.1% vs 66.1% at 4 weeks (p=0.054); 2.14% vs 48.3% at 12 weeks (p<0.05)). In addition, the recipient mice transplanted with CLEC2^low^ HSCs kept high chimeric levels of EGFP^+^ CMP and M^{K}P, whereas similar levels were detected in the recipients with CLEC2^high^ HSCs. On the other hand, CLEC2^high^ HSCs yielded 2.5-fold more M^{K}P than CLEC2^low^ HSCs in short-term grafts (1 to 2 weeks after transplantation) (p<0.05). Consistent with this finding, CLEC2^high^ HSCs yielded more CD41^+^ platelets than CLEC2^low^ HSCs by 6.0-fold at 1 week after transplantation (p<0.05), which peaked 10 weeks earlier than in CLEC2^low^ recipient mice. These platelets yielded through the transplantation of EGFP^+^ M^{K}P donor cells were detected at a certain level 12 weeks after transplantation. Furthermore, treatment with fostamatinib (R788), a Syk kinase inhibitor that is an indispensable component for CLEC2 signaling, blocked more potent and rapid megakaryopoiesis in the CLEC2^high^-recipient, indicating that CLEC2 signaling is essential for rapid and enhanced megakaryopoiesis from CLEC2^high^-donor HSCs.

Summary/Conclusions: Here, we showed that CLEC2 expression on HSCs demonstrates their oscillation for serving as a potent source of megakaryopoiesis, and found that CLEC2/Syk signaling would be involved in differential regulation between CLEC2^high^ and CLEC2^low^ HSC subtypes.
Background: GATA4 is a transcription factor expressed in mesoderm and endoderm during development. Members of the family such as GATA1-3, but not GATA4, are critically involved in hematopoiesis. An enhancer (G2) of the mouse Gata4 gene directs its expression throughout the lateral mesoderm and the allantois, beginning at E7.5, becoming restricted to the septum transversum by E10.5, and disappearing by midgestation (Rojas et al., Hepatology, 2005, 132:3405). Our previous work has shown that inactivation of Gata4 using this G2Cre driver is lethal by midgestation (Delgado et al., Hepatology, 2014, 59:2358). The anemia observed in the G2Cre;Gata1floX/floX 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 from G2Cre;R26RYFP embryos 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-Gata4flox/flox;R26RYFP mice, adults and embryos, by flow cytometry, RT-PCR and confocal microscopy. Cells obtained from different tissues were cultured and transplanted to analyze in vitro and in vivo potential.

Results: YFP+ cells represented about 20% of the hematopoietic system of adult mice and contributed in the same proportion to the lymphoid, myeloid and erythroid lineages. Adult YFP+ hematopoietic stem cells (Figure 1) constituted a long-term repopulating, transplantable population. Fetal YFP+ hematopoietic progenitors were much more abundant in the placenta than in the yolk sac area. 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. 1Hematology and Rheumatology, Tohoku University Graduate School of Medicine, Sendai, Japan

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 showed similar physiological behavior in normal mice, but this finding raises a number of questions, for example: Does this hematopoietic stem cell subpopulation show a differential response in physiopathological conditions? Does this subpopulation show a differential profile of gene expression? Does a similar heterogeneity exist in human HSCs? We are currently investigating the transcriptome of the G2-GATA4 lineage HSC in order to answer these questions.

E1104

EXPLORING THE MECHANISM OF FOGL-DEPENDENT TRANSCRIPTIONAL REGULATION IN ERYTHROID CELLS

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Background: GATA-1 is a hematopoietic zinc-finger transcription factor, which controls the development of erythrocytes and megakaryocytes. Numerous proteins have been reported to be associated with GATA-1 to alter its activity. Among them, Friend of GATA-1 (FOG1), a nine-zinc finger protein, is expressed in a tissue-specific pattern that overlaps markedly with that of GATA-1. FOG1 is an essential co-regulator of GATA-1 during hematopoiesis, which mediates transcriptional activation and repression of GATA-1 target genes; yet, the mechanisms by which FOG1 exerts its activating and repressing functions remain unknown.

Aims: We explored a novel role of FOG1 by forcibly expressing FOG1 in the human K562 erythroleukemia cells.

Methods: GFOG1 mRNA was cloned into Flexi HaloTag vector (Promega) and pBabe-puro retrovirus vector (Addgene), and FOG1 was overexpressed in K562 cells. Quantitative ChIP analysis was performed using antibodies against GATA-1, GATA-2, TAL1, FOG1, histone H3 acetylated-K4 (H3K4ac), H3K9ac, H3 trimethylated-K4 (H3K4me3), and H3K27me3. PU.1 regulatory element was cloned into luciferase plasmid (pGL4.10, Promega) and mutation within the cis-element was introduced using a site-directed mutagenesis kit (Agilent). TAL1 loss-of-function analysis was conducted with specific siRNA. For transcription profiling, Human Oligo chip 25K (Toray) was used.

Results: Forced FOGL1 expression in K562 cells induced the expression of erythrocytes genes (HBA, HBB, and SLC4A1), whereas repressed that of GATA-2, which have been reported to be FOGL1-dependent GATA1-target genes (Lee et al, Mol Cell 2009). On the other hand, FOGL1 overexpression did not affect the expression of master regulators of erythropoiesis, such as GATA-1 and TAL1. Next, we conducted microarray analysis to comprehensively characterize FOGL1-regulated gene signature. The analysis demonstrated that 942 and 180 genes were upregulated and downregulated (>2-fold), respectively, in the FOGL1-overexpressed cells. Noticeably, we found that the expression of PU.1, known as a myelo-lymphoid-promoting transcription factor, was strongly downregulated by FOGL1 overexpression, indicating that PU.1 is another FOGL1-dependent target. Aims: To explore the role of FOG1 in the regulation of GATA-1-regulated genes and suggest that FOG1 has an important role in inducing cells to differentiate toward the erythroid lineage rather than the myelo-lymphoid one by repressing the expression of PU.1.

E1105

THE STEM CELL ZINC FINGER 1 (SZF1) / ZNF589 PROTEIN INHIBITS TUMOR DEVELOPMENT IN A K562 XENOGRAFT MOUSE MODEL, BLOCKING CELL CYCLING AND INDUCING PREMATURE CELLULAR SENESCENCE

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Background: The stem cell zinc finger 1 (SZF1) / ZNF589 protein, a member of the family of Krüppel associated box-domain-zinc finger (KRAB-ZNF) transcription factors, has an isoform exclusively expressed in CD34+ hematopoietic stem/progenitor cells (HSPC) suggesting its role as an epigenetic regulator of specific genes involved in hematopoiesis. The SZF1/ZNF589 gene exhibits a human-specific evolutionary nucleotide DNA-change leading to a complex molecular organization and to a protein structure peculiar for humans, as compared to all other primates, potentially conferring human-specific functional properties. SZF1/ZNF589 has recently been shown to control cell viability in the hematopoietic system. It is regulated by the HIF-1a-hypoxia-induced transcription factor and is differentially expressed in a cytokine-dependent manner during hypoxia in CD34+ HSPCs (Venturini et al., Exp Hematol 2016; 44: 257-268). Thus, SZF1/ZNF589 may play a role in the maintenance of hematopoietic progenitors, lymphocyte quiescence and survival, known to be influenced by the hypoxic state in the bone marrow niche.

Aims: We studied the effects of SZF1/ZNF589 overexpression in vitro and evaluated its tumor suppressor potential in vivo.
Methods: K562 (BCR-ABL-positive chronic myeloid leukemia in blast crisis)-Luciferase-control or K562-Luciferase-SZF1/ZNF589 cells were directly injected into the femurs of NSG mice and tumor development was monitored by bioluminescence. Furthermore, K562 cells with or without SZF1/ZNF589 overexpression were studied by proliferation assay, cytometry, flow cytometry, cell cycle analysis, cyclin B1 expression and beta-galactosidase assay.

Results: K562-dependent tumor growth was efficiently inhibited in NSG mice transplanted with K562-Luc-control-cells, leading to significantly prolonged survival, demonstrating a strong tumor suppressive potential of SZF1/ZNF589 in vivo. In vitro, overexpression of SZF1/ZNF589 dramatically inhibited proliferation of K562 cells, which instead of dying, became giant and dysplastic, without other significant morphological changes and in absence of polyplody. Cell cycle analysis revealed a blockade in G2/M phase, with cyclin B1 accumulation characteristic for mitotic arrest. As suggested by morphology and beta-galactosidase assay, these results were consistent with premalignant transformation.

Summary/Conclusions: SZF1/ZNF589 controls survival of hematopoietic cells mediated by mitotic arrest and premature senescence, exhibiting tumor suppressive functions in vivo.

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 use of lineage-specific DNA patterns. It is of linearity 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 DNA 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 knockdown of Tr.1+3+4 (Tr.1+3+4). Global DNA methylomes were generated with the Infinitium HumanMethylation450 BeadChip platform and gene expression patterns with the Human Affymetrix Genome U133 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 DNA patterns: several CpG sites revealed significant differences in DNA methylomes upon knockdown of Tr.2 and Tr.1+3 (5,905 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 DNA methylomes. Furthermore, modulation of DNMT3A splice variants resulted in transcript-specific gene expression changes, which may at least partly be attributed to DNAm 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 DNAm changes. Thus, alternative splicing of DNMT3A is relevant for site-specific epigenetic modifications in hematopoietic development.

E1107 ERYTHROPOIETIN STIMULATES TRANSDIFFERENTIATION OF BONE MARROW PRO-B CELLS INTO BONE-RESORBING OSTEOCLASTS

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Background: Erythropoietin (EPO) is a crucial kidney-derived hormone responsible for erythropoiesis; however, its extra-erythroid effects are substantial and correlate with EPO receptor (EPO-R) expression in both hematopoietic and non-hematopoietic tissues. Bone turnover is regulated by the coupled actions of osteoblasts, the bone-forming cells, and monocyte-derived osteoclasts, which mediate bone resorption. In this regard, we have recently reported that EPO directly stimulates bone resorption via activation of EPO-R signaling in the monocytic lineage (Hiram-Bab et al., 2015). Monocyte differentiation into osteoclasts relies on monocye-macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor kappa B ligand (RANKL). B cells are also known to regulate bone metabolism, chiefly via paracrine signals. Osteoclasts and B cells arise from distinct myeloid and lymphoid progenitors, respectively, which are downstream of a common multipotent progenitor cell.

In the bone marrow (BM), Pro-B cells sequentially differentiate into Pre-B and immature B cells. Whether BM B cells can transdifferentiate into osteoclasts remains controversial, since osteoclast differentiation from residual mononuclear precursors in the cultures was not excluded in earlier studies.

Aims: We set to determine whether B cells can transdifferentiate to osteoclasts and to assess the effect of EPO on this process.

Methods: Experiments were conducted on C57BL/6j or CD19-Cre;R26R-EYFP, 8-12-week-old female mice in accordance and with the approval of the Institutional Animal Care and Use Committee of Tel-Aviv University (M-14-043). BM cells were flushed from femurs, ilias, and pelvic bone and red blood cells were lysed. Cells were stained with labelled anti-mouse antibodies: PE-B220, FITC-CD19, PerCP-igm, PeCy7-CD43, and APC-M-CSF receptor/CD115; and sorted by flow cytometry. Cells were then cultured in α-MEM containing 10% fetal bovine serum, M-CSF and RANKL. Multinucleated osteoclasts were stained for tartrate-resistant acid phosphatase (TRAP) and pit resorption was assessed.

Results: B cells isolated from BM of CD19-Cre;R26R-EYFP mice cultured with M-CSF and RANKL differentiated into TRAP+ multinucleated osteoclasts that were also positive for EYFP, thus tracing back their B cell origin (Figure 1A).

Next, we dissected which B cell subtype possesses this osteoclastogenic capacity and found that only Pro-B (B220+CD19+CD43+highGM), but not Pre-B (B220+CD19+CD43-owGM) nor immature B cells (B220+CD19+CD43+owGM) could transdifferentiate into osteoclasts (16%±3.7 from 0.79%±0.28 and 48%±13 osteoclasts’ area, respectively). Moreover, among the Pro-B cells, only those expressing M-CSF receptor (CD115) could transdifferentiate into osteoclasts (18%±6.55 vs. 0.11±0.05 osteoclasts’ area, respectively, Figure 1B and C). Using an anti-EPO-R specific antibody we detected EPO-R on the surface of B cells and noted that EPO enhanced the differentiation of the Pro B cells into osteoclasts by as much as 70% (p=0.04) (Figure 1D).

Figure 1: Osteoclastogenesis in vitro from sorted B cells. (A) Transdifferentiation of 180,000 cells/well CD19-Cre;R26R-EYFP into osteoclasts. (B) TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. (C) Right – Pro-B cells not expressing CD19-Cre;R26R-EYFP into osteoclasts. (D) TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (C) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (C) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (C) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (C) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05.
EXTN011

BONE MARROW MYELOPOIESIS INDEPENDENTLY OF CANONICAL NOTCH SIGNALING

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Background: Notch signaling is a highly conserved pathway important in multiple developmental processes. Canonical signaling through all Notch receptors converges on the CSL transcription factor recombination signal binding protein for immunoglobulin kappa J region (Rbpj). In haematopoiesis, Notch is crucial 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 myeloid (Mk) and erythroid (E) development as well as in suppressing granulocyte-macrophage (GM) progenitor expansion and acting as a tumor-suppressor in myeloid malignancies. However, these findings were largely made through genetic approaches potentially also affecting regulatory pathways and further studies are needed.

Aims: To unambiguously investigate the role of canonical Notch signaling in aBM myelopoiesis, in steady-state and following transplantation.

Methods: B6-SJL/JCD45.1, Rbpjfl/fl, Mx1-Cre, Vav-Cre and Vwf-eGFP BAC mice were used. Flow cytometry (FACS) was applied for phenotypic analyses. Gene expression levels were measured by real-time reverse transcription-PCR. Mk and GM in vitro colony forming potentials were applied in mouse colony assays. For transplantation study, lethally irradiated recipients were competitively transplanted (1:1) and reconstituted assessement 7-9 weeks after transplantation.

Results: FACS staging of GM, Mk and E progenitors in aBM of D2f-flanked Rbpj mice crossed to both Mx1-Cre and the pan-haematopoietic Vav-Cre strains was applied. As expected, HSCs were unaffected. Not previously investigated, FACS analyses did not identify GM, Mk or E progenitor stages. However, defects, at any progenitor stage, in Rbpj-deficient mice. To demonstrate that this lack of a phenotype was not due to BM cells escaping Rbpj deletion, we FACS purified HSCs and all GM, Mk and E progenitor stages from Rbpj-deficient mice and verified a virtually complete deletion of Rbpj in all populations. In further agreement with canonical Notch signaling not being required for steady-state generation, maintenance or stepwise differentiation of adult GM, Mk and E progenitors, the number of GM, E and Mk colonies generated from unfractionated aBM cells as well as circulating platelet counts were also unaffected. Our results suggest that Notch signaling is not essential for the emergence of definitive hematopoietic stem cells (HSCs) in the embryo and in thymic T cell development. However, in contrast to the previous studies, our findings are the first to demonstrate that canonical Notch signaling is dispensable for HSC homeostasis in the adult bone marrow. We next sought to address whether we could uncover a role of the Notch pathway in regulation of GM, Mk and E progenitors in aBM and in the bone marrow niche. However, in a significant minority of patients these LSCs could therefore be of significant clinical benefit. One of the proposed mechanisms of drug resistance in CML LSCs is close contact with the surrounding microenvironment, however an in-vitro model of the bone marrow matrix is currently lacking.

Conclusion: Development of a 3-Dimensional culture using fibre scaffolds to mimic bone marrow microenvironment is necessary to study the mechanism of resistance to anti-leukaemia agents.

Methods: Scaffold production: PMMA solution was prepared by dissolving PMMA in chloroform and adding appropriate amount of hydroxyapatite to polycaprolactone. 3D cell culture: A single-cell suspension of primary AML cells and also MSCs were added to PMMA-HA scaffold. K562 and HL60 cells were treated with or without imatinib or doxorubicin respectively. 3D cell culture: The same experiment was performed in the presence of scaffolds. Co-culture of HS-5 with HL60 or K562 cells: GFP+ stromal cells were added to PMMA-HA scaffold. K562 and HL60 cells were treated in the presence or absence of imatinib or doxorubicin in HS-5 cells plus scaffold environment respectively and were investigated for proliferation and viability 72h later.

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 or absence of cytotoxic or targeted therapy to that of cells grown in 2D culture. PMMA-HA scaffold was not toxic to the leukaemia cells as primary AML cells and also K562 cells grew in the presence of scaffold and also concentrated around the scaffold.
fibrillar collagen type 6. 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 cell HS-5 to the scaffold reduced even further the sensitivity of K562 or HL60 to imatinib or doxorubicin, respectively.

![Figure 1.](image)

**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 in vivo model to study the mechanisms involved in leukemogenesis.

**Aims:** We report two cases of donor cell derived haematological malignancy in which whole-exome sequencing (WES) was performed in bone marrow (BM) samples from recipient at different times after allogeneic 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-DS).

**Methods:** Case 1: A 43-year-old female diagnosed with lymphoblastic leukemia/lymphoma (11;19), who developed acute myeloid leukemia (AML) with normal karyotype. NPM1+ of donor origin 16 months after unrelated cord blood transplantation (UCBT). Case 2: A 65-year-old male diagnosed with mantle cell lymphoma, who developed MDS 45XX;−7.del(12)(p12) of donor origin, 57 months after allo-

genetic BM transplantation from his HLA-identical brother. WES (SureSelect-XT Human-exon 50M) was performed by next generation sequencing (HiSeq) on donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT. The exome of donor SCs and 5 BM samples, from case 1, were aligned to the human reference genome (GRCh 37/hg19) and donor SCs and 9 BM samples were aligned to GRCh 38/hg38 in the second case.

**Results:** WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukemia in bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukemogenesis. (Case 1: SH2B3 and case 2: KMT2C, KMT2A, ARHGAP26 and monosomy 7). Somatic mutations, acquired over time, fall into genes that play well-established roles in signalling pathways. Mutations in leukemic subclones that disappear after chemotherapy were indentified, as well as the acquisition of new mutations in resistant subclones. We propose a possible model of leukemogenesis in these cases (Figure 2).

**Summary/Conclusions:** The present study reveals a process of sequential clonal expansions, possibly by acquisition of additional somatic mutations in donor hematopoietic cells. Detection of heritable or acquired gene mutations in donor associated with predisposition to haematological malignancies could have clinical implications for the patients undergoing allo-HSCT. Although the cause of donor cell derived haematological malignancy onset seems to be multifactorial, the infusion of a SCU pre-leukemic potential in a context of residual toxicity in recipient as a result of pre-transplant chemotherapy, a post-transplant environment characterized by a decreased immune surveillance may well have played role in these cases. The study of a greater number of DCL cases by next generation sequencing could help to understand this process and to detect new mutations involved in the emergence of AML.

E1114 LEUKEMIC STEM CELL-RELATED MRNA EXPRESSION ANALYSIS USING A NOVEL FLOW CYTOMETRY-BASED ASSAY

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**Background:** Gene expression analysis of protein-coding (mRNA) and non-coding RNA in paediatric and adult acute myeloid leukaemia (AML) has become of paramount importance for therapeutic decision-making, revealing prognostic information and for the identification of novel therapeutic targets. AML is a clinically, phenotypically and molecularly heterogeneous haematological malignancy, with different leukemic cell populations organized in a hierarchical fashion, and leukemic stem cells (LSCs) residing at the apex herein. Unfortunately, gene expression profiling is commonly performed on unfractionated bulk samples, leading to ‘expression averaging’ of these heterogeneous cell populations. Multicolor flow cytometry (FCM) is capable of distinguishing heterogeneous cell populations based on the phenotypic characterization at a single-cell level. However, fluorochrome-conjugated antibodies are not available for intracellular RNA targets.

**Aims:** To evaluate the applicability of a novel flow cytometry-based technique, PrimeFlow™ RNA assay, to measure cell-of-interest RNA expressions in heterogeneous AML samples.

**Methods:** Technical assessment was performed using six neuroblastoma cell lines with varying levels of MYCN gene amplification. Correlation to expression data obtained by the gold standard RT-qPCR, performance in rare (0.1%) cell populations, effects of cryopreservation and off-target effects were evaluated. Next, diagnostic material of de novo AML patients was used to measure target gene (Wilms’ tumor 1 (WT1)) and reference gene (RPL13a, GAPD) expression. Expression analysis was performed in unfractionated bulk leukemic cells as well as blasts and rare subsets of leukemic cells, e.g., LSCs. FMCC analyses were performed on a FACSCanto II (BD Biosciences) with set-up according to EuroFlow guidelines. Infinicyt™ (Cytognos®) was used for data analysis and mean fluorescence intensities (MFI) values (with/without normalisation) were interpreted. P-values < 0.05 were considered significant.

**Results:** mRNA expression quantified by PrimeFlow™ significantly correlated with data obtained by RT-qPCR and remained detectable in rare (0.1%) cell populations. WT1 expression was shown to be statistically significantly higher in bulk leukemic cells of those patients characterized by WT1 overexpression, as defined by RT-qPCR, showing a mean 52% MFI upregulation by PrimeFlow™, with data obtained by the gold standard RT-qPCR. Moreover, WT1 overexpression could be detected within heterogeneous cell populations, e.g., the CD34+CD38+ cell population and the LSC (defined as CD34+CD38−), showing a 63% and 45% MFI upregulation, respectively. WT1 overexpression could be detected in leukemic subclones that disappear after chemotherapy were indentified, as well as the acquisition of new mutations in resistant subclones. We propose a possible model of leukemogenesis in these cases (Figure 2).

**Figure 1.**

**Figure 1.**

**Methods:** Case 1: A 43-year-old female diagnosed with lymphoblastic leukemia/lymphoma (11;19), who developed acute myeloid leukemia (AML) with normal karyotype. NPM1+ of donor origin 16 months after unrelated cord blood transplantation (UCBT). Case 2: A 65-year-old male diagnosed with mantle cell lymphoma, who developed MDS 45XX;−7.del(12)(p12) of donor origin, 57 months after allo-

genetic BM transplantation from his HLA-identical brother. WES (SureSelect-XT Human-exon 50M) was performed by next generation sequencing (HiSeq) on donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT. The exome of donor SCs and 5 BM samples, from case 1, were aligned to the human reference genome (GRCh 37/hg19) and donor SCs and 9 BM samples were aligned to GRCh 38/hg38 in the second case.

**Results:** WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukemia in bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukemogenesis. (Case 1: SH2B3 and case 2: KMT2C, KMT2A, ARHGAP26 and monosomy 7). Somatic mutations, acquired over time, fall into genes that play well-established roles in signalling pathways. Mutations in leukemic subclones that disappear after chemotherapy were indentified, as well as the acquisition of new mutations in resistant subclones. We propose a possible model of leukemogenesis in these cases (Figure 2).

**Summary/Conclusions:** The present study reveals a process of sequential clonal expansions, possibly by acquisition of additional somatic mutations in donor hematopoietic cells. Detection of heritable or acquired gene mutations in donor associated with predisposition to haematological malignancies could have clinical implications for the patients undergoing allo-HSCT. Although the cause of donor cell derived haematological malignancy onset seems to be multifactorial, the infusion of a SCU pre-leukemic potential in a context of residual toxicity in recipient as a result of pre-transplant chemotherapy, a post-transplant environment characterized by a decreased immune surveillance may well have played role in these cases. The study of a greater number of DCL cases by next generation sequencing could help to understand this process and to detect new mutations involved in the emergence of AML.
E1115

POTENTIAL PREDISPOSING GERMLINE MUTATIONS IN PATIENTS WITH CONCOMITANT MYELOID AND LYMPHOID MALIGNANCIES

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Background: Recent findings have suggested that mutations predisposing the development of either acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL) may arise in pre-leukemic hematological stem cells. In addition, genes involved in epigenetic regulation, such as TET2, and RNA processing, such as SF3B1, are mutated in both myeloid and lymphoid malignancies. This could indicate a possible genetic link between myeloid and lymphoid malignancies. Therapy related AML (t-AML) is a known complication to treatment with cytotoxic drugs such as alkylating agents and topoisomerase inhibitors. The susceptibility of developing t-AML has been associated with variation in DNA-repair pathways, drug metabolism and transport.

Aims: In this study, we aimed to investigate a possible common genetic origin of hematological cancers in patients with concomitant CLL and de novo AML or myelodysplastic syndrome (MDS) and in patients with concomitant therapy-related AML (t-AML) and CLL.

Methods: The presence of concomitant lymphoid and myeloid malignancies in patients is rare, however we managed to include 3 patients with de novo AML and CLL, one patient with MDS and CLL, one patient with chronic myelomonocytic leukemia(CMML) and CLL, and two patients with t-AML and CLL. The patients’ diagnoses were based on the evaluation of the morphology, immunohistochernistry, cyogenetics, and flowcytometry analysis in accordance to the WHO classification. For each patient mononuclear cells (MNCs) from blood or bone marrow were isolated using Ficol 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) mRNA analysis and cell software. Variants with a frequency of 5% or above were called.

Results: We identified possible pre-disposing germline mutations in all 7 patients by comparing variants between the myeloid malignant clone, CLL cells, and T cells, as well as using saliva to aid in characterizing the mutations as present or absent in the hematological compartment. In all the patients except one with de novo AML and CLL, we identified a potential damaging germline variant in a DNA-repair related gene, such as ATM (387dupA, D130fs*4), SMARCAL1 (2114D>T, T705S), HELQ (393_397delAGGTG, T1226*16), SWI5 (652C>T, R218*), LIG1 (2168A>G, Q761R) and PRKDC (2102G>A, G331Y). In the remaining patient with concomitant de novo AML and CLL, we identified a potential damaging germline variant in an epi-genetic regulator believed to play a role in normal and malignant hematopoiesis, KDM2B(44delC, P15fs*92). Furthermore, we identified the somatic mutational landscapes of the malignant clones using T-cells as germline tissue for the somatic mutational landscapes of the malignant clones.

Summary/Conclusions: Analysis of the mutational landscape of CHIP has clearly highlighted the role of DNMT3A mutations in concomitant hematopoiesis in older healthy individuals, the significance of such preleukaemic clones is yet to be determined.

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 concomitant hematopoiesis in older healthy individuals, the significance of such preleukaemic clones is yet to be determined.

E1117

NEXT-GENERATION REFERENCE INTERVALS FOR PEDIATRIC HEMATOLOGY

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Background: Interpretation of hematology analytes in children is challenging due to extensive changes in hematopoiesis with age leading to pronounced sex- and age-specific dynamics. To facilitate clinical decision making based on quantitative 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.
Figure 1.

Methods: We applied a data-mining algorithm to generate percentile charts for hematology analytes using laboratory data collected during the clinical care of patients. A total of 9,517,245 samples from 343,463 patients (72,614–337,011 samples per analyte) from 8 German tertiary care centers and 2 German laboratory service providers were examined. Percentile charts were calculated using an established statistical approach which extracts the proportion of samples from healthy individuals from the unfiltered input dataset containing both non-pathologic and pathologic samples. To evaluate the clinical benefit of hematology test result interpretation using percentile charts, accuracy and speed of pediatricians assessing eight different predefined clinical situations were measured in comparison to conventional test result representations.

Results: We created percentile charts for hematology analytes in girls and boys from birth to 18 years which can be used as common reference intervals. Results are provided for hemoglobin, hematocrit, red cell indices, red cell count, red cell distribution width, white cell count, and platelet count, example charts for hemoglobin, mean corpuscular volume, and platelet count are shown in the accompanying figure. A web application at www.pedref.org/hematology demonstrates hematology test result interpretation using percentile charts and z-scores with special consideration of pediatric dynamics. Comparison of pediatricians’ decision times when assessing different clinical scenarios using percentile charts and conventional representations shows more correct decisions (75.9% vs 68.4%, p<0.01) which are made in shorter time (2.7 s vs 3.8 s, p<0.01) when using percentile charts.

Summary/Conclusions: The created percentile charts enable the appropriate differential diagnosis of changes in hematology analytes due to disease and changes due to physiological development. Integration of suitable forms of result reporting using the provided percentile charts into clinical decision making improves assessment of the unique dynamics in pediatric hematology.

E1118

GROWTH FACTOR INDEPENDENCE 1 (GFI1) REGULATES THE AML SUPPORTING FUNCTION OF MESENCHYMAL STROMAL CELLS

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Background: Mesenchymal stromal cells (MSCs) harbor and support the function of normal hematopoietic stem cells. Less is known about their interaction with leukemic cells, e.g. in acute myeloid leukemia (AML). The prognosis of AML, a clonal malignant disease of the bone marrow (BM), is still poor with only 25% of patients living longer than 5 years.

Aims: In the current study, we investigated the interaction between MSCs and AML cells, and we also investigated the underlying molecular mechanism.

Methods: We used cell cultures using primary cells from human and mice and cell lines of MSCs and AML cells. Different Mouse models of human AML were used in our study to confirm the results obtained from human sample. MSCs were characterized by differentiation assay, flow cytometry and RT-PCR. Matrigel test was also applied in this study.

Results: MSCs from AML patients called AML-associated MSCs (AMSCs) or from murine models of human leukemia enhance significantly in vitro the growth of leukemic cells compared to AML cells growing without MSCs or in presence of MSCs from non-leukemic patients or mice. Among other, AMSCs increased entry of leukemic cells into the cell cycle, and at the same time protected the leukemia cells against exogenous toxic events such as chemotherapy or irradiation. The interaction between AMSCs and leukemia cells is dependent on cell-to-cell contact. In vivo, absolute and relative numbers of AMSCs and other stromal cells, i.e. endothelial cells and osteoblast lineage cells were highly expanded in the BM of mice modeling of human AML. AMSCs showed a higher efficiency of capillary tube formation in the matrigel assay than normal MSCs which gives an additional indication that AMSCs were polarized by leukemia cells towards a tumor-supporting state. On a molecular level, the polarization of MSCs towards an AML-supporting state depends on upregulated expression of the transcription factor Growth factor independence 1 (Gfi1). Loss of Gfi1 diminished the tumor-supporting state of AML-associated MSCs.

Summary/Conclusions: We conclude that leukemia cells polarize AMSCs towards a leukemia-supporting state in a Gfi1-dependent manner, which could open the way to new therapeutic approaches.
BASELINE LEUKOCYTE AND EOSINOPHIL COUNTS PREDICT OUTCOME IN RELAPSED OR REFRACTORY CLASSICAL HODGKIN LYMPHOMA PATIENTS TREATED WITH PD1 INHIBITION

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Background: Despite encouraging efficacy of anti-PD1 antibodies in relapsed or refractory (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 as 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<0.0001). Only one PSF event occurred in the best prognostic group (n=10, median PFS (days): 365 [129-NA]) and 7 out of 9 patients in intermediate group (median PFS (days): 197 [50-NA]). Evaluation of best response achieved according to the initial risk score showed a trend towards higher CR-rates in low risk group, but was not significant.

Figure 1.

Summary/Conclusions: Our simple prognostic model, mainly characterized by a normal to high REC, robustly discriminates three risk groups for PFS. Almost all patients in the low risk group achieved a durable remission without disease progression throughout the study period, despite often achieving just a partial response. In contrast, high-risk patients often progressed quickly despite initially achieving a partial or complete response. Further validation of this score which is easily available from routine clinical parameters in a larger cohort of patients and further investigation of its potential predictive impact is needed. Moreover, efforts to clearly understand a possible mechanistic role of eosinophils in cHL patients treated with PD1-inhibition are warranted.

THE PROGNOSTIC SIGNIFICANCE OF BETA-2 MICROGLOBULIN (B2M) LEVELS IN PATIENTS WITH HODGKIN LYMPHOMA (HL) TREATED WITH ABVD OR EQUIVALENT (ABVDEQ) CHEMOTHERAPY OR COMBINED MODALITY THERAPY (CM) IN ELDERLY PATIENTS

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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 important in NCCN and ESHO-EBMT guidelines. CS stage needs to be defined in order to avoid overtreatment. Moreover, efforts to clearly understand a possible mechanistic role of B2M in HL are needed. In this context, the aim of this study was to explore the prognostic significance of serum b2m levels in HL patients treated with ABVD or ABVDEQ chemotherapy or combined modality therapy (CM).

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/MT (1990-2016) and selected solely based on the availability of pretreatment b2m levels. B2m [P1] levels (upper normal limit 2.4mg/L) were analyzed according to other baseline features and prognostic factors as well as according to the outcome. Freedom From Progression (FFP) was defined as time between treatment initiation and treatment failure (primary refractoriness, PR with switch to alternative CT or relapse); deaths of unrelated causes were censored. Overall Survival (OS) was measured from treatment initiation to death of any cause. ROC curves and sequential cut-offs (1.8-3.5 by 0.1 increments) were used to explore the potential impact of b2m on FFP and OS.

Results: The median follow-up for currently living patients was 88 months. Univariate Analysis: FFP was significantly inferior in patients with higher b2m at all tested cut-off points. At 2.4mg/L (normal versus elevated) the 10-year FFP was 81% vs 71% (p=0.003). However, the best cut-off was the observed median value of this series, calculated at 2.1mg/L, with 10-year FFPs 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-off values yielded statistically significant results (borderline at 2.0mg/L vs 1.9mg/L; 77% vs 67%, p=0.057). Multivariate Analysis: B2m levels remained significant for FFP after adjustment for IPS factors, ESR and B-symptoms at both 2.1mg/L and 2.4mg/L cut-offs (hazard ratio (HR) 1.78, p=0.001 and 1.41, p=0.04 respectively) in the whole series of 864 patients. In early stages, b2m was a significant predictor of FFP at the cut-offs of 1.9mg/L and 2.1mg/L (HR 2.00, p=0.01 and 1.83, p=0.02 respectively), but only borderline at the cut-off of 2.4mg/L (HR 1.65, p=0.07). In advanced stages, b2m was emerged as an independent prognostic factor for FFP at the cut-off of 2.2mg/L (HR 1.59, p=0.046 despite the lack of significance in univariate analysis), but was not significant at the 2.4mg/L cut-off. The 10-year OS was lower in patients with high b2m levels (10-year rates 91% vs 76%, p<0.0001).

Summary/Conclusions: Higher serum b2m emerged as a significant independent predictor of FFP for cut-offs of 2.0mg/L for the whole series and 1.9mg/L for early-stage patients. The prognostic significance in advanced stages was weaker (best cut-off 2.2mg/L). Serum b2m was also highly predictive of OS. This is by far the largest report on the prognostic significance of b2m in HL, highlighting the significance of the cut-off used to define “high” levels. Its significance is more pronounced in early stage disease. The optimal cut-off for the evaluation of serum b2m in HL may be stage-dependent and appear to lie between 1.9 and 2.2mg/L, thus performing better than a “normal versus high” evaluation (cut-off 2.4mg/L).

THE PREDICTIVE VALUE OF INTERIM PET-CT IN ELDERLY PATIENTS WITH HODGKIN LYMPHOMA

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Background: 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, a 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 OS, 6 (7%) achieved PR, 10 (11%) were primary refractory and 2 (2%) died during treatment. Fifteen (16%) patients experienced relapse. Five years PFS and OS were 56% and 78%, respectively. ABVD treated patients had 5 year PFS and OS of 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. Fifty 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 PFS and OS were 82% and 85%, respectively. The 5 year PFS of these patients differed according to the depth of response on iPET - 69% vs 45%, (p=0.02, fig.1) in patients achieving NED vs PR, while 5 year OS did not reach statistical significance, 90% vs 71% (p=0.08). Restricted analysis, evaluating only 59 patients who were treated with ABVD, showed clinical results with 54% of NED iPET vs 45% of PR iPET achieving NED on EOT-PET (p<0.01). Outcome differed according to the depth of response in iPET with 5 year PFS rates of 74% vs 34%, in patients achieving NED vs PR, respectively (p=0.01). 5 year OS rates were 92% vs 76%, in patients achieving NED vs PR (p=0.1).

E1122
HIGH-DOSE BENDAMUSTINE PLUS BRENTUXIMAB COMBINATION IS EFFECTIVE AND HAS A FAVOURABLE TOXICITY PROFILE IN THE TREATMENT OF REFRACTORY AND RELAPSED HODGKIN LYMPHOMA
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Background: The management of patients with refractory or relapsed Hodgkin lymphoma (HL), especially after autologous stem cell transplantation (ASCT), remains controversial. Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the schedule in patients with HL, in particular its dosage and the possible combinations for a synergistic effect. Brentuximab Vedotin is a CD30-directed antibody-drug conjugate, currently approved for the treatment of relapsed or refractory HL.

Aims: The objective of this retrospective observational trial was to evaluate efficacy and safety of salvage cytotoxic regimens in patients with refractory and/or relapsed HL. Three different regimens were evaluated.

Methods: From May 2011 to December 2016, 32 consecutive patients (19 M/13 F) with a median age of 31.7 years (range, 16-73) received a salvage regimen after failure of ASCT. Patients were by chance assigned to one of these three arms: standard dose bendamustine (90mg/sqm) days 1 and 2 plus standard DHAP schedule (every 4 weeks) x 3 cycles (Arm A, n= 10 cases), brentuximab single agent 1.8mg/kg (every 3 weeks) x 4-8 cycles (Arm B, n= 11 cases), high dose bendamustine (120mg/sqm) days 1 and 2 plus brentuximab 1.8mg/kg (day 3) x 4-6 cycles (Arm C, n= 11 cases). Each cycle in arm C was repeated every 28 days and growth factor support was systematically administered, in association with antimicrobial prophylaxis. The treatment efficacy in each arm was evaluated according to Revised Response Criteria for Malignant Lymphoma by Cheson et al. Adverse event occurrence was recorded and classified and type and grade using NCI-CTCAE criteria (v 4.0).

Results: In arm A, the overall response rate (ORR) was 40% (4/10 patients), with 4 (40%) complete remission (CR) and 6 (60%) progressive disease (PD). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (40%) and bone marrow aplasia in 1 patient (10%); extra-hematological toxicity was gastrointestinal toxicity of grade 2 in 6 patients (60%) and grade 1 in 3 patients (30%), in arm B, ORR was 63.6% (7/11 patients), with 5 (45%) CR, 2 (18%) partial response (PR) and 4 (36%) PD. Hematological toxicity was grade 2 neutropenia in 4 patients (36%), extra-hematological toxicity was grade 3 neuropathy in 2 patients (18%). In arm C, ORR was 100% (11/11 patients), with 11 CR followed by SCT (second autologous transplant, 6 cases; and haploidentical transplant, 5 cases) with persistence of complete remission in all patients at a median follow-up of 33.4 months (range, 12-60). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (36.3%); extra-hematological toxicities were increase of transaminase (grade 2) in 7 patients (27%) and cytomegalovirus (CMV) reactivation in 2 patients (18%), treated successfully with valganciclovir. Three patients had fever during infusion at first cycle, together with a skin rash, managed with corticosteroid injections, and a successful antihistamine plus corticosteroid prophylaxis in the next cycles of treatment.

Figure 1.
Summary/Conclusions: High-dose bendamustine plus brentuximab has shown relevant efficacy and a relatively good safety profile in a setting of heavily pretreated patients with HL. Adequate monitoring of CMV reactivation is recommended. This combination could be considered as a bridge to second autologous or allogeneic SCT. However, these results should be validated by controlled and prospective studies involving larger number of patients.

E1123
NEED OF HORMONAL THERAPY TO PRESERVE FEMALE FERTILITY IN HODGKIN E NON-HODGKIN LYMPHOMA PATIENTS FOLLOWING CHEMOTHERAPY: A TWO-CENTER SURVEY
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Background: In the last decades, Hodgkin and Non-Hodgkin Lymphoma (HL-NHL) therapies have resulted in high cure rates and increased survival. However, the young patients (≤50 years), experienced more late toxicities such as, gonadal toxicity that can result in permanent infertility.

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 (pregnancies, 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) was consider to be 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 [EST] (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 significant difference between the regularity of menses and the event of an abortion pre and post treatment. As for pregnancies, 35% of patients had children before therapy and 17% after. Among these 109 patients, 68/109 (62%) received gonadotropin-releasing hormone (GnRH) analogues and/or oral contraception, while 41 (38%) were not treated with hormonal therapy. Among the 68 patients who received hormonal therapy regular menses recovered in 61/68 (90%) while in those of the control group a recovering 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 (8/69, 12%) compared to 30/60 (50%) patients that had regular menses after chemotherapy, 61/81 (75%) received hormonal therapy and 20/81 (25%) were not treated with hormonal therapy. Before treatment for lymphoma, 16% of patients belonging to the hormonal group had pregnancies versus 45% of the control group (P<0.05). Following therapy, pregnancies were observed in 23% of those receiving hormonal therapy vs 5% of the control group (P<0.05).

Summary/Conclusions: The use of hormonal therapy is fundamental not only to favor of pregnancies and motherhood but in particular to avoid the consequences of an irregular cycle or an early menopause with its symptoms and clinical implications.

E1124
25(OH) VITAMIN D SERUM LEVELS IN HODGKIN LYMPHOMA
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Background: Vitamin D has pleiotropic effects on cellular differentiation, proliferation, apoptosis and angiogenesis in addition to maintaining serum calcium and skeletal homeostasis. Several studies suggest that low serum 25(OH)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 2015; 33:1482]. 25(OH)D 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 33 years), diagnosed at our Institution between 2014 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 25 (33.3%) patients, and deficient in 43 (57.2%) 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=0.06). No association was found with gender, albumin level, B symptoms. In addition, there was a significant seasonal variation, with 25(OH)D levels being 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 Vitamin 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.
stem cell transplantation (ASCT). Patients treated with R alone or R+ABVD had better DFS (p=0.04) than those treated with ABVD with/without IFRT. Specifically, the year Kaplan-Meier estimates for DFS were 100% for the R treated group versus 50% for those treated with ABVD with/without IFRT. Four patients in the latter group, showed insufficient response to the therapy: 1 refractory disease in the early stage group and 3 recurrent diseases in the advanced stage group were recorded. The median follow-up time of the entire cohort of patients was 4.3 years (range, 0.5-8.2 years). Over the study period, one patient died for infectious pneumonitis due to severe neutropenia following the last cycle of R-ABVD. Of the 9 patients treated with addition of IFRT, adverse events regarded mainly thyroid (4), bone (2), lung (1) and salivary glands (1). Nobody developed a secondary malignancy.

Summary/Conclusions: Our results confirm the value of R in NLPHL and show that R induction and maintenance combined with chemotherapy only in the presence of risk factors or in more advanced stages give excellent treatment results. Proper risk assessment and understanding of treatment options in the pre- and post-transplant setting are critical to ensure optimal longer progression-free survival for qualified patients.

Aims: Underlying clinical practice gaps and educational needs were identified, and a study was conducted to determine whether an online, case-based educational intervention could improve knowledge, competence, and confidence of hem/oncs in managing patients with HL.

Methods: The educational format presented patient case scenarios (2) followed by a series of 4-5 questions that “tested” learner knowledge and competence before delivering the education focused on the optimal approach to the case using evidence-based medicine. Case questions assessed degree of patient risk for disease relapse or progression prior to ASCT and consolidation strategies, taking into consideration patients’ prior received therapies. To assess educational effectiveness, participants served as their own controls by responding to a set of the same questions again after (post-assessment) exposure to the content. For all questions combined, the McNemar’s chi-square test assessed differences from pre- to post-assessment. P values are shown as a measure of significance; P values <0.05 are statistically significant. Cramer’s V calculation determined the change in proportion of 184 participants who answered questions correctly from pre- to post-assessment and who qualified for the study.

Results: At post-assessment, there was a large effect to the education (V=0.442), indicating a sizable improvement in evidence-based choices and significant improvement in knowledge, competence, and confidence related to managing patients with HL, including: 138% relative improvement regarding the implications of type and number of prognostic factors on risk of HL relapse and benefit of consolidation brentuximab vedotin after ASCT (P<.001); 101% relative improvement in knowledge that a higher rate of relapse after ASCT is associated with a CR duration of less than 1 year, extranodal disease at relapse, and the presence of symptoms at relapse (P<.001); 5% relative improvement in knowledge regarding the efficacy of brentuximab vedotin in relapsed/refractory HL after ASCT (P<.001); Responses to a self-efficacy question indicated that 42% of hematologists became more confident in managing a patient on consolidation therapy for HL after participating in the education.

Summary/Conclusions: This study demonstrated the success of an online, case-based format using a predisposing pre/post-assessment was effective in improving the evidence-based practice patterns of hem/oncs in the management of patients with HL. Despite the marked improvement in knowledge, competence, and confidence, hematologist education needs specific to accurate risk assessment, treatment selection, and adverse effect monitoring remain. The education gaps uncovered during this intervention and the evolving treatment landscape outside of the United States lay a foundation for future global education initiatives to bridge education gaps in HL.
**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, G. Hess et al. Blood 2013). Here, we describe the effect of ibrutinib treatment on T-cells 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., ≥2 prior lines of therapy, and progressive disease [PD]) ≤12 months after last dose of a CIT regimen. All pts received ibrutinib (560mg QD) on a 21-day cycle until PD or unacceptable toxicity. The primary end point was Independent Review Committee (IRC)-assessed overall response rate (ORR) (complete response [CR] + partial response [PR]). Flow cytometry assessed T-cell 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 A et al. ASH 2016). Briefly, 110 pts with a median age of 61.5 years and a median of 3 prior therapies were enrolled. Ibrutinib achieved an ORR of 20.9% (CR rate, 10.9%) and a median duration of response of 19.4 months. Flow cytometry analysis revealed significant downregulation of CD4+CD25+CD127- Tregs at C3D1 in 14 responders (CR + PR, mean decrease 17 to 12.9% CD4, 52% CD25, 41% CD127, p<0.0025) and in 43 non responders (p=0.035, compared to responders). From a large panel of inflammation-related cytokines and chemokines, some of the most significant changes at C2D1 were the Th1 cytokines interferon-γ (IFN-γ) and interleukin (IL)-12, both of which were increased in responders but decreased in nonresponders (p=0.0025 and p=0.035, respectively, Figure 1). Conversely, the chemokines IFN-γ-induced protein 10 (IP-10) and monocyte-chemotactic protein 3 (MCP-3) were decreased in responders but increased in nonresponders (p=0.022 and p=0.016, respectively).

**Summary/Conclusions:** Here we show immunomodulatory effects of ibrutinib in pts with CIT refractory FL, which may be related to response to therapy. In responding 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 chemotraction of T-cells and monocytes/macrophages. These data suggest that immunomodulatory effects of ibrutinib could play a role in its antitumor activity in FL, so combinations with other immune-oncology therapies may prove beneficial.

**E1130**

**DYNAMO: THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH DOUBLE-REFRACTORY SMALL LYMPHOCYTIC LYMPHOMA IN A PHASE 2 STUDY**

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**Background:** Duvelisib is an oral, dual inhibitor of PI3K-δ,γ in development for the treatment of hematologic malignancies. DYNAMO is a Phase 2 study to evaluate the safety and efficacy of duvelisib in a double refractory indolent NHL 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:** DYNAMO is an open-label, single-arm, safety and efficacy study in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL), whose disease is double refractory to rituximab (monotherapy or in combination) and to chemotherapy or radioimmunotherapy. Pts received duvelisib 25mg BID in 28-day treatment cycles until disease progression or unacceptable toxicity. The primary endpoint is overall response rate (ORR) as assessed by an independent review committee (IRC) per revised IWG criteria. Secondary endpoints include duration of response (DoR), progression-free survival (PFS), overall survival (OS), time to response (TTR), adverse events (AEs), and changes in safety laboratory values. *Pneumocystis jirovecii* pneumonia (PJP) prophylaxis was mandated for all pts at baseline.

**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 an ECOG performance status score at baseline of 0 (43%), followed by 1 (54%) and 2 (4%). Most SLL pts had either low (51%) or intermediate (49%) disease burden. 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 un evaluable 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 cytope尼亚s (neutropenia [23%], anemia [12%], and thrombocytopenia [10%]), and diarrhoea (15%). 4 SLL pts had SAEs that led to discontinuation. Duvelisib: NSCLC neuronal toxicity, cutaneous toxicity of the skin, pseudomembranous colitis, and pneumonia. Two SLL pts has a fatal AE, 1 pneumonia and 1 viral infection.

Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory SLL population (68% ORR, median DOR 9.9 mo., 93% with a reduction in target lesions). Duvelisib was generally well tolerated, with a manageable safety profile with appropriate risk mitigation. Duvelisib monotherapy appears to have a favorable benefit-risk profile in double refractory SLL. Updated clinical data will be available at the time of presentation.

E1131
Abstract withdrawn.

E1132
WALDENSTROM MACROGLOBULINEMIA: UK REAL WORLD EXPERIENCE
D. El-Sharkawi1, H. Renshaw1, M. Lunn2, D. Hughes3, A. Rismanli2, S. D’Sa1
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Background: There are few randomised controlled trials in Waldenström macroglobulinemia (WM) due to its rarity and indolent nature. As a result, there is no standard treatment approach and management is variable.

Aims: The aim of this retrospective study was to review “real world” management of WM in the UK and correlate this with survival outcomes.

Methods: All patients with a diagnosis of WM seen at ULCH between 01/07/2002 and 31/12/2016 were included. Patient characteristics, presenting features, lines of treatment, responses and overall outcome were recorded. Survival was estimated using Kaplan-Meier analysis from time of first treatment and P values calculated using the log-rank test.

Figure 1. Results: A total of 211 patients were identified (116 M/ 95 F), median age 60 yrs (range 34-89). Presenting symptoms included anaemia, n=53; neuropathy, n=19; fatigue, n=18; hyperviscosity symptoms, n=13; lymphanepthony, n=5; progression from IMUS, n=5; B symptoms, n=5; other, n=28; unknown, n=55. Mutated MYD88 was seen in 59 of 72 cases analysed (82%). Of these 59 cases, 13 were CXCR4 mutated. IPSSWM was known in 150 cases of whom 64 were in low, 63 intermediate and 23 high risk groups. Median follow-up from first appointment was 64 months (range 0-394). The median number of lines of therapy was 2 (range 0-9). Dexamethasone, rituximab and cyclophosphamide (DRC) was given to 62 patients upfront, 52 had other cyclophosphamide containing regimens e.g. CHOP +/- rituximab, 29 had Chlorambucil-based regimen, 14 R-bendamustine, 15 fludarabine-based with a minority getting R-cladribine (5) or R-bortezomib (4), 9 pts had no treatment at all. Notably, DRC was given to 1 patient before 2009, 28% of patients between 2009 and 2013, and 41% from 2013. In the 149 cases with known responses to first line treatment, 11% achieved a CR (7 patients with R-CHOP, 4 DRC, 2 fludarabine containing regimen, and 3 patients other treatment). 63% PR/VGPR, 21% no response or PD and 5% stopped due to toxicity. For the 52 patients who had DRC chemotherapy, median PFS was 61 months. Of those patients who had at least 3 lines of chemotherapy (n=62), median time between 1st and 2nd line treatment was 42 months, 3 months between 2nd and 3rd line. Transplants were performed on 28 patients after a median of 2 lines of chemotherapy. Median overall survival (OS) has not been reached in the 195 patients with available data. Stratifying by IPSSWM shows median OS for the low risk group has not been reached, 11 years for the intermediate risk and 9 years for the high risk group. P=0.29 (Figure). Patients had a significantly reduced OS if they developed Binet Neel syndrome or high grade transformation compared to other known complications of WM. Despite differences in chemotherapy strategies over the past two decades, there was no difference in outcome in patients treated...
WALDENSTRÖM MACROGLOBULINEMIA (WM). A STUDY ON 114 OFF OF THERAPY.

S. Guidez1, J. Labreuche 2, J. Bakala 3, B. Royer 4, C. Delette 4, M. Joris 4,

PATIENTS (PTS) treated with WM have been followed up for ~6 years after diagnosis. After ~6 years of follow-up: 63.6% (Rx4+RIT), 66.7% (R-COP+RIT) have not progressed by consolidation with 90Y-IT remains as a valid option for follicular lymphoma. Risk of second progression rate after 1st line therapy was 70%. Sixty-two, 37 and 19 pts received a 2nd or 3rd and 4 to 6 lines of therapy respectively according to the 2nd International Workshop guidelines. Monitoring of serum monoclonal immunoglobulin concentration (SMIC) throughout the evolution of the disease was available in 106 pts. Informed consent was obtained according to the protocol submitted to the Institution Review Board.

Results: Median survival after 1st line therapy was estimated 79 months. It was estimated 69 and 65 months after 2nd line and 3rd line therapy respectively. High IPSSWM (hiPSSWM vs low/intermediate) retained prognostic value for survival after 1st treatment initiation (SAFTI, p=0.0005). However, plot of hazard function showed a decrease of hazard ratio over time with a departure from the proportional hazard hypothesis (Grambsch and Therneau test: p=0.053). Consequently, Dxy concordance index obtained in multiple landmarks analyses decreased from 0.27 to 0.12, during the first 6 years of follow-up. In Cox model of SAFTI with time dependent covariate, onset of response (whatever cut-off in SMIC) had no prognostic value. Onset of progression and initiation of 2nd line therapy, retained prognostic values for SAFTI (p=0.0038 and p=0.004 respectively). Only 2 thresholds in SMIC defined a response status (observed between onset of response and progression) of prognostic value for SAFTI: namely >25% reduction in SMIC (i.e. minor response or better; p=0.041) and >50% (i.e. partial response or better; p=0.026). In similar Cox models with hiPSSWM, onset of progression (p=0.0034) and 2nd treatment initiation (p=0.0031) retained independent prognostic value beside hiPSSWM (p=0.0026). Times elapsed from the initiation of 1st line therapy to 1st progression and to the initiation of 2nd line therapy had no prognostic value for subsequent survival. In similar Cox model of survival after 2nd line therapy with time dependent covariate no threshold in SMIC were found to be associated with a significant value of onset of response or response status. Neither onset of progression nor next treatment initiation had significant prognostic value. Similar results were observed after the 3rd line of therapy.

Summary/Conclusions: The prognostic value of initial ipPSSWM decreased in part during the first 6 years of evolution. Onset of progression and 2nd treatment initiation provided additional prognostic information for predicting SAFTI. Therefore progression-free survival or time to next treatment may be satisfactory surrogate endpoint of SAFTI in WM. Further international collaborative studies are mandatory for this purpose. Assessing response in more advanced phase of the disease may require specific tools.

Figure 1.

Summary/Conclusions: The use of immunotherapy with rituximab or combined schedules with immunotherapy (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+R) of patients continue with complete response and off of therapy.

ASSESSING RISK OVER TIME IN PATIENTS WITH SYMPTOMATIC WALDENSTRÖM MACROGLOBULINEMIA (WM). A STUDY ON 114 PATIENTS. S. Rupoli1,*, G. Goteri2, G. Micucci1, I. Federici1, L. Canafoglia1, P. Leoni1, L. Bugatti7, S. Serresi4, R. Santilli4, A. Campanati3, N. Pimpinelli8

Background: Development of cut-off in SMIC (i.e. >25% and >50% reduction in SMIC) in the second line therapy to define the response status and progression was investigated in a study on 114 patients with WM treated in our institutions. The prognostic value of initial ipPSSWM decreased in part during the first 6 years of evolution. Onset of progression and 2nd treatment initiation provided additional prognostic information for predicting SAFTI. Therefore progression-free survival or time to next treatment may be a satisfactory surrogate endpoint of SAFTI in WM. Further international collaborative studies are mandatory for this purpose.

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). The follow-up of these protocols was proportional and combination was yearly published (Rupoli et al., EJD 2016). The follow-up of these protocols was prolonged up to February 2017.

Methods: We recruited patients with stages I-IV MF who had failed PUVA (early disease) or several systemic regimens (early and advanced disease). We defined high "min" and "standard" protocols in which Bexarotene dose 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.

E1136 TIME TO NEXT TREATMENT ANALYSIS FOR EARLY AND ADVANCED STAGES OF MYCOSIS FUNGOIDES/SEZARY SYNDROME TREATED WITH BEXAROTENE AND PUVA IN COMBINATION. S. Rupoli1,*, G. Goteri2, G. Micucci1, I. Federici1, L. Canafoglia1, P. Leoni1, G. Rondinelli1, F. Giannomasso1, G. Moscicchio1, R. Albertini1, M. Saninacci1, L. Bugatti1, S. Serresi1, R. Santilli1, A. Campanati3, N. Pimpinelli8

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Assessing response in more advanced phase of the disease may require specific tools.

Background: Bexarotene is a synthetic retinoid effective in early and advanced stages of Mycosis Fungoides (MF)/Sezary Syndrome (SS) both in monotherapy and combination schemes. Time to next treatment (TTNT) seems to be a clinically meaningful endpoint that incorporates both symptom control and disease progression. It has been investigated in few retrospective studies focusing on retinoids in monotherapy both in limited-stage and advanced stage MF, but up to now no data are available concerning the use of retinoids in combination.

Aims: To assess the prognostic role during the clinical course of initial international prognostic index (IPSSWM), response and progression (according to 6th International Workshop guidelines). Monitoring of serum monoclonal immunoglobulin concentration (SMIC) throughout the evolution of the disease was available in 106 pts. Informed consent was obtained according to the protocol submitted to the Institution Review Board.

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). The follow-up of these protocols was proportional and combination was yearly published (Rupoli et al., EJD 2016). The follow-up of these protocols was prolonged up to February 2017.

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E1135 TIME TO NEXT TREATMENT ANALYSIS FOR EARLY AND ADVANCED STAGES OF MYCOSIS FUNGOIDES/SEZARY SYNDROME TREATED WITH BEXAROTENE AND PUVA IN COMBINATION. S. Rupoli1,*, G. Goteri2, G. Micucci1, I. Federici1, L. Canafoglia1, P. Leoni1, G. Rondinelli1, F. Giannomasso1, G. Moscicchio1, R. Albertini1, M. Saninacci1, L. Bugatti1, S. Serresi1, R. Santilli1, A. Campanati3, N. Pimpinelli8

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Methods: We recruited patients with stages I-IV MF who had failed PUVA (early disease) or several systemic regimens (early and advanced disease). We defined high "min" and "standard" protocols in which Bexarotene dose 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.
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.

Aims: To characterize the clinical-physiological characteristics and to determine the prognostic impact of blood involvement in patients with advanced FL.

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 IIIB and 1 with stage IVA). Six patients had previously received PUVA therapy only, while fifteen patients had received other therapies. The protocol proved to be effective, well tolerated and able to induce an overall response of 55.6% at the end of induction phase (93.4% of early stage patients and 66.6% of advanced stage patients) and of 76.2% at the end of maintenance phase (86.7% of early stage patients and 14.2% of advanced stage patients). Median follow up for all patients was 85 months (6-118) with respectively 98 months (21-118) for early stages and 46 months (6-102) for advanced stages. For the entire cohort, median OS, PFS and TTNT were 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 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.

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 immunophenotype. 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 wait approach (7% vs 9%), type of treatment, or overall response rate (83% 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 group 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 second 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.

E1137 PERIPHERAL BLOOD INVOLVEMENT IN PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA: CLINICAL-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT

A. Rivas-Delgado1,*, L. Magnano2, P. Mozas1, I. Dlouhy1, J. Rovira1

Aims:

Background:

Results:

Methods:

Background:

Results:

Methods:

Table 1.

Table 1. Table of results of patients with FL treated with a ≥NCI-recommended therapy

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Figure 1.
Background: The antipaptotic protein BCL-2 is commonly overexpressed in hematologic malignancies. VEN is a potent, selective, orally bioavailable BCL-2 inhibitor that has demonstrated acceptable safety and antitumor activity in NHL and MM patients.

Aims: To evaluate the safety, PK profile, and preliminary antitumor activity of single-agent VEN in NHL patients with or without prior exposure to chemotherapy.

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 was performed on 21-day cycles until progression. All pts received tumor lysis syndrome (TLS) prophylaxis and underwent routine tumor evaluation, 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. Responses were assessed by 2007 IWG (NHL) or 2006 IWG (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 1200-mg dose cohort had proarrhythmia, which was suspected to be due to DLT as DLT occurred following 2 doses of 100-mg VEN on day 2 of the dose ramp-up period. One DLBCL pt died while on study due to disease progression. No TLS events were reported. Steady-state VEN exposures were nearly dose proportionally across 300-mg to 900-mg doses. At the 1200-mg dose, exposures to VEN increased less than dose proportionally, which is consistent with non-Japanese subjects. VEN exposures were comparable between Japanese and non-Japanese pts at the 300-mg dose. At higher doses, individual exposures were generally within the range observed in non-Japanese pts but mean exposures were 30–100% higher. Overall, the OR rate was high, with nearly half the pts with NHL achieving an OR. Further evaluation of VEN in Japanese pts with hematologic malignancies is ongoing.

E1139

A SIMPLIFIED APPROACH IN THE ASSESSMENT OF T-CELL CLONALITY BY FLOW CYTOMETRY

A simplified approach for the clinical evaluation of abnormal T-cell populations by flow cytometry. "In summary we have demonstrated a novel approach in the assessment of T cell clonality by targeting T-cell receptor β constant domain 1 (TRBC1). The addition of Jovi-1 in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.

E1140

A HIGHER AMOUNT OF LILOTOMAB PRE-DOSE INCREASES THE ACTIVITY-ADJUSTED AUC AND HAS A PROTECTIVE EFFECT AGAINST MYELOSUPPRESSION OF LUTETIUM (177Lu)-LILOTOMAB SATETRAX-IM-MYXELDIO-177Lu, A SIMPLIFIED APPROACH IN THE ASSESSMENT OF T-CELL CLONALITY"
of prior therapies ranged from 1 to 7, median body weight was 79 kg (range: 58-118kg). The administered activity across all treatment groups ranged from 746 to 1982 MBq. The table below shows the summary of the median PK and haematology safety results for $^{177}$Lu-lilotomab by treatment group. The activity-adjusted AUC$_{0-\infty}$ of $^{177}$Lu-lilotomab increased with 100mg/m$^2$ of lilotomab compared to the other pre-dosing regimens (p<0.001 compared to 40mg lilotomab). The median volume of distribution and clearance were both reduced with 100mg/m$^2$ of lilotomab compared with the other pre-dosing regimens. However, activity adjusted Cmax was similar. Smaller percentage post-treatment reductions in platelet and neutrophil counts were observed in patients receiving 100mg/m$^2$ lilotomab. Most common grade 3/4 AEs were hematological and were transient and reversible.

**Table 1.**

![Table 1](image)

Summary/Conclusions: A higher pre-dose of lilotomab 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 lilotomab 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 lilotomab pre-dosing is ongoing and will be presented.

E1142

**PHARMACOKINETICS AND TOLERABILITY OF OFATUMUMAB AND BENDAMUSTINE IN PATIENTS WITH INDOLENT B-CELL NON-HODGKIN’S LYMPHOMA**

A. Forero-Torres1,*, J.C. Chandler2, S.P. Iyer3, A.S. Kanate4, M. Quinlan5, P. Hoeve6, M. Izquierdo6, V. Duval6, S. Madan7

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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 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 Cmax, AUC$_{last}$, AUC$_{inf}$ were derived using non-compartmental analysis. All adverse events (AEs) and severe AEs (SAEs) were recorded for safety assessments.

Results: Thirty two patients were randomized (15 in Arm A and 17 in Arm B), 3 patients in Arm A discontinued study treatment due to consent withdrawal (2 patients) and infusion related AE (1 patient). All 32 patients were included for safety and PK concentration analysis while 30 patients (15 in each arm) were included for PK parameters. Patient and disease characteristics were similar between treatment arms; the majority of patients from both arms did not receive prior NHL therapy. PK concentration profiles and PK parameters of OFA were comparable when administered alone or co-administered with BEN (Table 1). As compared to OFA alone, there was a decrease of 14% in Cmax and 15% in
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 Han 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 a 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 (86.4% of those with only a FBC performed) and 64(88.9%) in those with a FBC and DWCC). The thrombocytopenia (platelets (Plt) <100x10^9/l) and anaemia (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 1/3 of these triggering the 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.

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, predominantly when the parasitism 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.

E1144

A PROSPECTIVE MULTICENTER STUDY OF CANDIDIASIS IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL DISEASES: INCIDENCE, RISK FACTOR AND OUTCOMES

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1E1143

Aims: To assess the performance of the CHBAH NHLS SR rules in the detection of malaria in the Chris Han Baragwanath Academic Hospital Laboratory (CHBAH) (part of the National Health Laboratory Service (NHLS) network) in JHB, South Africa.

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 a 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.

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E1145

BRONCHOALVEOLAR LAVAGE AS SYSTEMATIC APPROACH FOR EARLY DIAGNOSIS OF LUNG INFILTRATES AND INVASIVE PULMONARY ASPERGILLOSIS IN HEMATOLOGIC PATIENTS: A PROSPECTIVE SINGLE INSTITUTION STUDY

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Background: The best diagnostic approach of lung infiltrates (LI) remains to be established. Despite bronchoscopy with bronchoalveolar lavage (BAL) appears to be useful for LI diagnosis, hematologists and thoracic surgeons often have suspicions in performing this procedure in the immunocompromised patient at high-risk of procedure-related complications. A proper diagnostic approach at LI seems to be particularly relevant in neutropenic patients and/or in patients with an unfavorable clinical response to broad-spectrum antibiotics, in which the cause of LI are often filamentous fungi, as Aspergillus spp. To date, there were stratified risk factor, targeting the 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 non response to broad-spectrum antibiotics were defined as new or persistent (> 48 h) fever without improvement in the cause of 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-glucan, serum PCR for CMV, BAL
E1146

ESCAPE DRUG-RESISTANT INFECTIONS IN HEMATOLOGICAL MALIGNANCIES. DARE TO REVIEW!  
C. Gentille Sanchez1,*, K. Sun1, P. Teegavarapu1, Q. Qian1, P. Mamta2, S. Wong2, I. Ibrahim1, L. Rice1, S.R. Pingali1, S. Iyer1  
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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 antibiotic treatment in 25 of these ones (76%), Twelve cases of probable IPA, according to standard criteria, were diagnosed. Seven cases of LI fulfilling the radiologic criteria for IPA, though presenting only a positive Aspergillus PCR on BAL, were detected and treated as probable IPA. One life-threatening post-procedure complication was observed.

Summary/Conclusions: BAL seems a safe approach for an early diagnosis of LI in hematologic patients. The assessment of a broad diagnostic panel allowed the detection of a putative agent in 65% of cases. Assessment of Aspergillus by PCR on BAL proved useful for probable IPA diagnosis.

Results: Out of 769 patients consecutively admitted in our ward, 85 had LI and 47 of them underwent BAL (total amount: 51 procedures). A causal agent of LI was detected in 33 cases (65%) allowing to modify the ongoing anti-microbial treatment in 25 of these ones (76%). Twelve cases of probable IPA, according to standard criteria, were diagnosed. Seven cases of LI fulfilling the radiologic criteria for IPA, though presenting only a positive Aspergillus PCR on BAL, were detected and treated as probable IPA. One life-threatening post-procedure complication was observed.

E1147

PROPOSED PEGIFLGRASTIM BIOSIMILAR CHS-1701 DEMONSTRATES PHARMACOKINETIC AND PHARMACODYNAMIC SIMILARITY TO MARKETED PEGIFLGRASTIM IN A RAT NEUTROPENIA 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 myelosuppressive 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 (CYP)-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-∞. In the bone marrow by analyzing 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: 99.6, 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.

Results: Out of 6017 patients with Hematological Malignancies, 660 patients with 684 malignant diagnoses were found; 235 had MDS, 174 had AML, 105 had ALL, 144 had CML, 39 had AL, 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 up by urinary tract infections (38.2%). Patients with MDS (36.6%) and AML (25.3%) were mainly affected. A prevalent resistance to levofloxacin was detected in gram positives (44.2%), and Pseudomonas (36.7%).

Summary/Conclusions: Hematological malignancies 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 compared to gram positives. A significant resistance to levofloxacin, a prophylactic antibiotic, was also noted. New strategies for reducing ESCAPE in MDS and AML are required. Further statistical review of this data set will be presented at the EHA Meeting, Madrid 2017.
Background: Neutropenic sepsis remains a leading cause of morbidity and mortality in both haematology-oncology and general oncology patients on cytotoxic chemotherapy. A retrospective study published in the Journal of Infectious Diseases described a beta-lactam monotherapy in suspected neutropenic sepsis, discouraging the use of aminoglycosides, in view of potential toxicities. Increasingly in clinical practice, it becomes evident that our patient population is incredibly heterogeneous and with the emergence of multi drug resistant strains of microorganisms, high-risk individuals need to be identified early and first line antimicrobial treatment regimens tailored according to patient factors alongside local antibiotic.

Aims: To retrospectively review appropriate antibiotic use, microbial identifications and antibiotic sensitivities amongst adult cancer patients with neutropenic sepsis. To identify if any patient or disease characteristics are associated with infection episodes that would support the upfront usage of aminoglycoside containing antibiotic treatment regimens.

Methods: A retrospective review of patients treated for neutropenic sepsis was conducted for the period between 1/4/2015 to 11/10/2016. Analysis of potential risk factors including primary disease, age, sex, treatment regimen, albumin, neutrophil and lymphocyte count to assess potential association with adverse outcomes.

Results: There were 116 episodes of neutropenic sepsis in 92 patients in this period. Of these, 61 were haemato-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%) received Tazocin and an aminoglycoside as first line antimicrobial treatment. Fourteen isolates demonstrated resistance, including 2 cases of stenotrophomonas maltophilia and 12 cases of enterobacteriaeace. 13 of the 14 resistant isolates were found in haemato-oncology patients. Nine of these cases were resistant to single agent Tazocin but sensitive to an aminoglycoside. The mean age of cases with resistance was 70.3 years (range 54.2 to 84.8 years). There was no difference in sex or degree of neutropenia/lipohypoplasia in the cases that contracted resistant bacterial strains compared to those that were culture negative. Of the 4 fatal cases with resistant bacteria, 3 had low albumin (mean 25.5g/L cf. mean of 34g/L in resistant bacteria cases surviving).

Summary/Conclusions: This retrospective analysis supports the use of combination antimicrobials up front as first line treatment in high-risk patients with neutropenic sepsis. The study has demonstrated that the patient cohort most at risk of developing drug resistant bacteriaeae 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 79.8% of haemato-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 haematoma-oncology patients early or up front consideration for the additional usage of an aminoglycoside is essential in order to optimize favorable outcomes in this population. From this study, the proposed risk factors of isolating resistant strains of bacteria leading to adverse outcomes would be aggressive haematological malignancies, receiving more intensive cytotoxic therapy, multiple lines of treatment and low albumin. Further analysis in a multi center trial will allow our patient population and side close collaboration between clinicians and microbiologists is essential in providing optimal antimicrobial therapy algorithms in neutropenic patients.

E1119
PRELIMINARY RESULTS FROM A LONG-TERM REPEAT DOSE TOXICITY AND TOXICOKINETIC STUDY OF ANF-RHO, A NOVEL ANTI-NEUTROPENIC FACTOR OR.

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Background: ANF-Rho is a novel polyethylene glycol-modified granulocyte colony stimulating factor that has biological and biological properties that produce a prolonged pharmacokinetic and pharmacodynamic profile as compared to pegfilgrastim (Neulasta®). As such, it has potential applications in chemotherapy induced neutropenia and chronic idiopathic neutropenia. These disorders require prolonged administration of G-CSF agents to treat neutropenia. There-
Background: *Pseudomonas Aeruginosa* (PA) is a gram negative, ubiquitous, opportunistic pathogen. Its intrinsic resistance to many antibiotics and the selective pressure exerted by empiric antimicrobial use, led to the emergence of MPA in hematological patients with high mortality and morbidity rates among infected immunocompromised patients (pts). Considering our MPA incidence of 9% in 2007/08, an outbreak developed at the HW of “Campus Bio-Medico” University Hospital of Rome, from 2008, despite the measures employed from the previous 2 years (health personnel sensitization, regular air and water filters changing, isolation precautions).

Aims: To describe the MPA outbreak occurred between 2009 and 2013.

Methods: Our HW, opened in 2007, is composed by 7 rooms, each with a private WC: 2 single, 1 double and 4 single, positive pressure, each with a filter/purification of the water and air system, a bedpan and water samples from bedpan automatic washers (BAW); phase C: room environmental disinfection and microbial decontamination with nebulized H2O2 solution added with silver cations; phase D: disposal of BAW, introducing the use of disposable bedpans and Planning an environmental sampling and disinfection program.

Results: On 04/2013 we revised retrospective study data: 82 pts carried bacterial isolates; of these, 48 (59%) had MPA, classified as colonisation in 13 pts (mainly detected on rectal swabs) and true infection in 35: 10 pneumonias (mainly detected on bronchial swabs), 9 bloodstream infections (mainly detected on central venous catheters), 2 peritoneal, 3 anorectal/perineal, 5 urinary tract, 14 bloodstream infections (mainly detected in blood samples), 48 (59%) had MPA classified as colonisation in 13 pts, 28 pts received carbapenems also combined with other anti-infectious drugs. When detecting positive blood cultures, all pts were treated by improving environmental measures. Pseudomonas contaminates and survives on many ecological niches, being continuously reintroduced in nosocomial settings. Our experience highlights the value of environmental and personal hygiene measures on MPA infections control.

**Table 1. MPA isolates and mortality rate after phase D.**

<table>
<thead>
<tr>
<th>Year</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteremia (%)</td>
<td>35</td>
<td>56</td>
<td>51</td>
<td>52</td>
</tr>
<tr>
<td>Infection (%)</td>
<td>26</td>
<td>18</td>
<td>20</td>
<td>29</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>0</td>
<td>0</td>
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Summary/Conclusions: We identified the contaminated water residue from BAW, a new source of MPA spread in our HW, getting a full outbreak control by improving environmental measures. Pseudomonas contaminates and survives on many ecological niches, being continuously reintroduced in nosocomial settings. Our experience highlights the value of environmental and personal hygiene measures on MPA infections control.

E11152

MONITORING VORICONAZOLE PHARMACOGENOMICS AND PLASMA CONCENTRATIONS IN THE TREATMENT AND PREVENTION OF INVASIVE FUNgal DISEASE FOR HEMATOLOGICAL PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: Voriconazole has been widely used in treatment and prevention invasive fungal disease for immunodeficiency hematological patients. And the voriconazole plasma drug levels were associated with its efficacy and toxicity. The hepatic cytochrome P450 isoenzyme 2C19 plays a wonderful role in voriconazole metabolism. However if CYP2C19 genetic polymorphism can result in voriconazole metabolism and drug plasma level in setting of Asian population especially in hematologic patients is unknown.

Aims: To evaluate the effect of CYP2C19 polymorphism on the voriconazole (VCZ) plasma concentration of patients with hematological disease and the value of voriconazole plasma concentration in the treatment and prevention of invasive fungal disease (IFD).

Methods: Between January to August 2016, 76 hematological patients who received voriconazole for the treatment or prevention of invasive fungal disease were enrolled in this study. The population CYP2C19 polymorphism of voriconazole were performed using PCR-Pyrosequencing. The trough plasma concentrations of voriconazole (C<sub>trough</sub>) was determined using high-performance liquid chromatography (HPLC).

Results: Genotyping for CYP2C19 polymorphic isozyme variations showed that 32 subjects (43.42%) for the CYP2C19 wild-type, 43 (56.58%) for the CYP2C19 variant phenotype. CYP2C19 polymorphism was identified among Chinese adult oncohematological patients, patients received voriconazole treatment in our center. Based on the genotype analysis, 45 subjects were identified as extensive metabolizers’ group for EMs (CYP2C19*1/*1), poor metabolizers’ group for IMS (CYP2C19*1/*3), and there was a significant difference between CYP2C19 values in the two groups (1.66±1.86ug/ml vs 3.30±2.35ug/ml, p=0.000). The C<sub>trough</sub> of the 45 patients were detected for 119 times totally. The medium of the C<sub>trough</sub> 45 hematological patients were described. Lack of response to therapy was more frequent in patients with voriconazole levels <1.5mg/L (23.5%) than those with voriconazole levels >1.5mg/L (30.8%) (p=0.000). Furthermore, the C<sub>trough</sub> value of patients with adverse events is higher than the others (3.21±±4.656ug/ml vs 2.17±2.14ug/ml, p=0.042).

Summary/Conclusions: The single-center study showed that the mutation of CYP2C19 was quite common in Chinese hematological patients. Patients with CYP2C19 wild-type phenotype are extensive metabolizers, their C<sub>trough</sub> of voriconazole are significantly lower than patients with CYP2C19 non-wild-type phenotype (poor metabolizers). Appropriate concentrations of voriconazole can improve the efficacy of therapy and safety outcome.
Iron metabolism, deficiency and overload

E1154

GLYCOSYLATED FERRITIN MEASUREMENT FOR SECONDARY HEMOPOYETIC SYNDROME DIAGNOSTICS

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Background: Hemopoyetic 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 refractory fever referred to antibacterial therapy and/or prolonged cytopenia and/or organ (lungs, CNS) involvement was established after the other possibilities 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), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine, INR, C-reactive protein (CRP), procalcitonin (PCT), total ferritin, and glycosylated ferritin percentage. Mann-Whitney U test and ROC-analysis were used for statistical analyses.

Results: No differences were found in sepsis and SHPS for ALAT, ASAT, ALP, LDH, and bilirubin levels. The difference of INR, CRP, PCT, creatinine levels was significant (p<0.01). The most substantial difference in SHPS and sepsis groups had serum concentrations of ferritin, triglycerides, level of ferritin glycosylation (p<0.01) (Table 1). According to ROC-analysis, the area under the curve for ferritin, triglycerides and percentage of ferritin glycosylation were 0.78, 0.82, and 0.92, respectively.

Table 1.

Summary/Conclusions: The most difference between sepsis and SHPS was observed for triglycerides, ferritin and percentage of glycosylated ferritin. Percentage of glycosylated ferritin fraction seems to be the most indicative, which may make it useful for SHPS diagnostics and its differentiation from sepsis.

E1155

SERUM HEPONIDIN QUANTIFICATION IN INFLAMMATORY BOWEL DISEASES

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Background: Inflammatory bowel diseases (IBD) include different intestinal pathologies, most common among them are Colitis Ucerosa (CU) and Crohn’s Disease (CD). Pathogenesis of IBD is still unclear, however they are multifactor diseases, with genetic and autoimmune compounds, in combination of environmental factors. One of IBD symptoms is iron deficiency anemia.

Aims: We aimed to search for connection between serum hepcidin quantification and anemia in IBD.

Methods: We included 64 patients with IBD - 29 with Colitis Ucerosa (CU), and 35 with Crohn’s Disease (CD). They were diagnosed in University “Aleksandrovka” hospital in Clinic of Gastroenterology. Their results were compared to age and gender matched healthy controls. Laboratory assessments were analyzed for included groups – iron, ferritin, CRP, IL-6 and hepcidin. AAS, nephelometrical, ELISA and statistical methods were used during analyzes and obtained results interpretation.

Results: 53% from our patients had with iron deficiency anemia (IDA) and low hepcidin concentrations (5.9±1.1 µg/L) compared to control group (19.9±2.8 µg/L). The IDAs and 11 of included patients had combination of chronic disease (ACD). Their hepcidin levels were increased (59.9±6.4 µg/L) in comparison to healthy controls (19.9±2.8 µg/L); P<0.001. In patients with ACD/IDA, quantified serum hepcidin correlates positively to increased IL-6 (r=0.758, P<0.005) and CRP concentrations (r=0.899, P<0.001).

Summary/Conclusions: Quantification of serum hepcidin levels in IBD patients might be a key element in diagnosis and treatment of anemia in these patients. Serum hepcidin levels are useful marker for differential diagnosis between iron deficiency anemia and combination iron deficiency anemia/anemia of chronic disease.

E1156

MUTATIONS IN YARS2 CAUSE CONGENITAL SIDEROBLASTIC ANAEMIA WITHOUT SHOWING EVIDENCES OF MYOPATHY AND LACTIC ACIDOSIS

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Aims: We aimed to report a new case with a different clinical presentation.

Methods: We have identified two novel variations in YARS2 gene using Next Generation Sequencing (NGS) panel containing 10 genes involved in congenital and acquired sideroblastic anemia.

Results: The proband is a young woman aged 24 where we have identified 2 novel variations in YARS2 gene. One pathogenic splicing mutation NM.001044362.2 c.[1104-10A>G], and a missense variation NM.001044362.2 c.608 G>T; NP_001035526.1. p. Ser203Ile located in the C-core catalytic domain of the mitochondrial tyrosyl-tRNA synthetase. None of these two variations were previously reported in public databases (ExAC, NCBI SNP, Ensembl). Clinical data from the patient showed marked sideroblastic anemia (Hb 91 g/L, 32% ring sideroblasts), but not signs of muscle weakness or lactic acidosis and congenital sideroblastic anemia (OMIM #610957, ORPHA 2598). Up to date in the literature it has been reported 9 families with 11 affected individuals with mutations in YARS2 gene and affected from MLASA2.

Summary/Conclusions: Here, we reported a patient with mutation in YARS2 gene showing congenital sideroblastic anemia but presenting neither lactic acidosis nor myopathy. Therefore, patients with defect in YARS2 gene may present with a less severe clinical manifestations only involving congenital sideroblastic anemia without other extra-hematopoietic defects. MLASA2 must be considered in patients presenting with only congenital sideroblastic anemia since early diagnosis and supportive therapy will be important to prevent complications.

E1157

IRON CHELATION DATA OF CONGENITAL DISSEYRHTHOPIA ANEMIA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: Congenital dyserythropoietic anemia (CDA) is a rare, genetically heterogeneous disorder characterized with ineffective erythropoiesis, and congenital malformations in certain types. Patients present with varying degrees of anemia and acidosis (lactic acid levels were 1.8-2.2 mmol/L; creatinine kinase 23 U/I, normal range: 23-170 U/I), as could be expected due to previously reported cases in the literature. Functional assays are on-going to confirm pathogenicity of the novel missense variation.

Summary/Conclusions: Here, we reported a patient with mutation in CDA gene showing congenital sideroblastic anemia but presenting neither lactic acidosis nor myopathy. Therefore, patients with defect in CDA gene may present with a less severe clinical manifestations only involving congenital sideroblastic anemia without other extra-hematopoietic defects. Chelation therapy may be considered in patients presenting with only congenital sideroblastic anemia since early diagnosis and supportive therapy will be important to prevent complications.
Results: Of these 11 patients, 7 were CDA type II. The median age of diagnosis was 12 months (3-144) months and male to female ratio was 7/4. Median transfusion requirement per year at previous year prior to initiation of chelation was 12 times (0-17). All of the patients were on chronic transfusion programme at initiation of iron chelation except for 2 (one receives occasional transfusion, and the other patient was on chronic transfusion programme but became transfusion independent after splenectomy). The median age at last visit was 70 months (32m-40 years). The median value of serum ferritin at initiation of iron chelators was 822 ng/ml. All of the patients were initiated deferasirox for iron chelation at a median dose of 24mg/kg/day (10-40) and the median chelation follow-up duration was 27 months (2-54 months). Three of the patients were evaluated with cardiac and hepatic T2* assessment prior to and by the end of 1 year of chelation and none of the patients were found to have cardiac iron loading at chelation initiation, whereas 2 had severe and 1 had moderate LIC values. In the subsequent assessment under chelation of these 3 patients all still had cardiac T2* values above 20 ms, whereas 1 had mild and 2 had moderate LIC values. Serum ferritin levels prior to initiation and by the end of 1 year were compared and the difference was found statistically insignificant.

Summary/Conclusions: Patients with CDA are at risk for iron loading and they need to be screened for the iron loading periodically. The prompt chelation in these patients prevent organ failure risks at long term including cardiac failure, cirrhosis and endocrinopathies.

E1158

ORAL IRON CHELATION FOR TREATMENT OF HEREDITARY HEMOCROMATOSIS IN CHILDREN

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Background: Hereditary hemochromatosis (HH) very rarely presents during childhood. The most common form of HH in children is Juvenile Hemochromatosis (JH), a rare genetic disorder inherited with an autosomal recessive manner, resulting from mutations in either the hemojuvelin (HJV) (type 2A) or the hepcidin (HAMP) gene (type 2 B). Early diagnosis and closely monitoring of iron overload indexes, namely, serum ferritin levels, transferrin saturation and tissue iron measurement by magnetic resonance imaging (MRI) are essential in order to prevent permanent organ damage and potentially life threatening complications (cirrhosis, diabetes mellitus, cardiac dysfunction, and hypogonadism). Therapeutic intervention in children with HH may be problematic, as erythropoiesis is invasive and may not be well tolerated in young children. Iron chelation therapy can be implemented as an alternative treatment to erythropoiesis.

Aims: The scope of this study was to evaluate the use of an oral iron chelation therapy in young children with HH.

Methods: 3 children (2 females and 1 male) were diagnosed with HH at the aged of 4, 6 and 8 years old, respectively, based on increased ferritin and transferrin saturation, and tissue iron measurement. Iron chelation therapy with deferasirox was initiated based on ferritin and tissue iron levels. All patients had normal serum vitamin B12 and folate levels, liver, thyroid and renal function tests. Pregnant women and patients with a history of blood transfusion within last 3 months and/or those with acute renal failure, anaemia of chronic disease, hypothyroidism, additional cytopenias and ferritin, Anaemia was defined according to the WHO recommendation. Cohort characteristics were defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

Table 1. Clinical characteristics of the patients.

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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: HH is very rare disorder in children, most frequently showed increased ferritin and transferrin saturation and tissue iron concentration. Iron chelation therapy with deferasirox was effective, and the difference was found statistically insignificant. Clinical characteristics of the patients were given in Table 1.

Figure 1.

Results: Hypersegregation was detected in 25 individuals with iron deficiency (45%) and 1 healthy control (5%). It was significantly more frequent in the IDA group (48.8%) than in the ID group (30.7%) [p<0.001]. After iron treatment 3 IDA patients' peripheral blood smear were checked and with normalization of iron parameters including serum iron, total iron binding capacity, ferritin, Anaemia was defined according to the WHO recommendation. Cohort characteristics were defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

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Figure 1.
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)-tcrolimus (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 pre-transplantation and post-transplantation.

Results: At 6 months 56 out of 61 (92%) were alive. Anemia was observed in 30 (49%) of them, with only 8 cases (13.1%) showing Hb level below 100 g/l. Microcytic anemia was diagnosed in 2 of them (3.3%). One patient showed an iron deficiency anemia as a bone marrow failure, and hepcidin levels were elevated in all patients with microcytic anemia. Anemia progressively improved with sirolimus tapering.

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>8.1</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>75</td>
</tr>
<tr>
<td>Serum ferritin (µg/l)</td>
<td>21</td>
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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 into account in this setting, as a rare cause of anemia. In case of microcytic anemia, the evaluation of iron parameters and hepcidin provides the diagnosis of this rare type of anemia.

E1162

ORAL IRON ELEVATES SERUM IRON AND CONSEQUENTLY CHANGES IRON DISTRIBUTION IN LIVER AND ERYTHROCYTES

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1Product Research Department, Chugai Pharmaceutical Co., Ltd., Kamakura, Japan

Background: For renal anemia patients, there are several therapeutic options including erythropoiesis-stimulating agents (ESAs), intravenous and oral iron supplementations. The sera of patients with non-renal hemosiderosis had significantly higher serum iron levels when compared with the sera of non-sick controls. The mechanism leading to this increase was not fully elucidated yet.

Aims: To load iron orally, a diet including 200 ppm of iron was used as control and a diet including approximately 5000 ppm of ferric citrate was used as iron-rich diet. 6-week-old male C57BL/6Ncr mice were divided into 3 groups; control group, intravenous iron (IV iron) group, and oral iron (Oral iron) group (n=5). Mice in IV iron group were fed a control diet from days 0 and intravenously administered 0.4mg/mouse of iron-dextran on days 9. Mice in Oral iron group were fed an iron-rich diet from days 0 and intravenously administered 0.4mg/mouse of dextran 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, iron levels were elevated in all groups and expression levels of duodenal DMT1 were significantly higher than control group. As for iron distribution in liver, iron was accumulated in reticuloendothelial cells in IV iron group, while 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 hemoglobin contents in each reticulo- 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 supplementations.
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. Patients 3 and 4 presented with neonatal anaemia requiring regular transfusion and were diagnosed with Pyruvate Kinase deficiency. Deferasirox was started at 12 and 19 months consecutively. 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 deferasirox 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 transplant at 7 years of age. Deferasirox was subsequently stopped.

Summary/Conclusions: All of these children had stabilization or improvement of ferritin values after initiation of deferasirox as shown in figure 1. Deferasirox was preferred in severe anaemias due to better side effect profile on the bone marrow compared to deferiprone; the use of which can cause agranulocytosis or neutropaenia. Furthermore, its oral administration improved compliance compared to deferoxamine that required prolonged subcutaneous administrations. Deferasirox has been associated with renal impairment. However, none of patients developed renal or liver impairment during the use of deferasirox. Furthermore, it is crucial to conduct eye and ear screening tests both before and after the commencement of deferasirox. None of our patients had neurological side effects. Three of these children had deferasirox started at younger than 2 years of age. Hence, we have shown that deferasirox is safe and efficacious in treating iron overload in very young children with rare anaemias and sickle cell disease where evidence is sparse.

Figure 1.

Summary/Conclusions: All of these children had stabilization or improvement of ferritin values after initiation of deferasirox as shown in figure 1. Deferasirox is licensed in Europe to be used in children with thalassaemia older than 6 years of age or older than 2 year of age when desferoxamine therapy is inappropriate or inadequate. Deferasirox is preferable in severe anaemias due to better side effect profile on the bone marrow compared to deferiprone; the use of which can cause agranulocytosis or neutropaenia. Furthermore, its oral administration improved compliance compared to deferoxamine that required prolonged subcutaneous administrations. Deferasirox has been associated with renal impairment. However, none of patients developed renal or liver impairment during the use of deferasirox. Furthermore, it is crucial to conduct eye and ear screening tests both before and after the commencement of deferasirox. None of our patients had neurological side effects. Three of these children had deferasirox started at younger than 2 years of age. Hence, we have shown that deferasirox is safe and efficacious in treating iron overload in very young children with rare anaemias and sickle cell disease where evidence is sparse.

E1164

MONITORING ORAL IRON THERAPY IN CHILDREN WITH IRON DEFICIENCY ANAEMIA. AN OBSERVATIONAL, PROSPECTIVE, MULTICENTRIC STUDY

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1Clinical and Experimental Medicine, University of Catania, 2Pediatric Hemato-Oncology Unit, 3Azienda Policlinico Vittorio Emanuele, Catania, 4Department of Pediatric and Public Health Sciences, University of Torino, Turino, 5Pediatric Hemato-Oncology Unit, University of Padova, Padova, 6Pediatric Hemato-Oncology Unit, IRCCS C. Mondino dell’Saronno, San Giovanni Rotondo, 7Pediatric Hemato-Oncology Unit, Policlinico S.Orsola Malpighi, Bologna, 8Pediatric Unit, Carlo Poma Hospital, Mantova, 9Department of Cellular Biotechnologies and Hematology, “Sapienza” University, Roma, 10Maria Paterno Areez Hospital, Ragusa, 11Hospital, Treviso, 12Central Teaching Hospital, Bolzano, 13Spedali Civili, Brescia, 14Ospedale Meyer, Firenze, Italy

Background: Iron deficiency anemia (IDA) is the most common hematological disease in infancy and childhood. Oral iron administration is a well-established, effective, and widely accepted treatment for anemia because of its efficacy, safety, and cost-effectiveness. Recently new preparations of oral iron compounds were launched, including droplet formulations, i.e. liposomal preparations and bis-glycinate iron; little is known on their effectiveness in real clinical practice.

Aims: To evaluate the efficiency of different oral iron preparations in children with IDA.

Methods: This observational study collected clinical and hematological data from 12 AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) centers. Inclusion conditions for patient enrollment were age 3 months-12 years, diagnosis of IDA; exclusion criteria were all conditions interfering with iron absorption such as celiac disease, gastro-intestinal disorders and other chronic conditions. Local Physicians were free to prescribe any oral iron formulation, according to their standard practice. A calendar of laboratory test was suggested, including basal assessment of whole blood count, reticulocytes, iron status, with subsequent checkpoints at 3 days (WBC and reticulocytes only), 2 weeks, 8 weeks, 6 months. Clinical data regarding compliance to therapy, unwanted effects, final outcome were recorded.

Results: 112 (M 58) patients were enrolled. Ethnic distribution was: Caucasian 74, African 23, Asian 10, Other 5. The median age at diagnosis of IDA was 1.5 years, with a bimodal distribution with frequency peaks at age 3 and 12-14 years. Sixty-eight patients received bis-glycinate ferrous iron 0.45mg/kg, 19 elemental iron (ferrous gluconate/sulfate) 2mg/kg, 12 liposomal iron 0.7-1.4mg/kg, and 15 other preparations. Eating habits were reported as normal in 48 patients, inadequate weaning in 21, meatfish restriction in 32, other in 11. Gastro-intestinal side effects were reported in 96/8 (13%) of the bis-glycinate iron group, in 5/19 (16%) of the elemental iron group, and in 5/12 of the liposomal iron group. Suspension of therapy due to side effects was needed only in 5 patients, 4 in the bis-glycinate and 1 in the elemental iron group, respectively. Final outcome was available for 77 patients; it was recorded as solved IDA, persistent IDA, or lost at follow up. Solved cases were 40/53 (75%) in the bis-glycinate iron group, 5/19 (26%) in the elemental iron group, and 8/12 (67%) in the liposomal iron group. Persistent cases were 8/53 (15%) in the bis-glycinate iron group, 6/11 (55%) in the elemental iron group, and 1/13 (8%) in the liposomal iron group. Lost at follow up were 5/53 (9%) in the bis-glycinate iron group, 1/11 (9%) in the elemental iron group and 4/13 (31%) in the liposomal iron group.

Summary/Conclusions: The collected data show that both bis-glycinate and liposomal iron formulations have a good efficacy/safety profile and offer a sustainable alternative to classic elemental iron preparations.

E1165

AN INVESTIGATION ABOUT WEIGHT GAIN WITH TREATMENT OF IRON DEFICIENCY ANEMIA: CHANGES OF GHRELIN AND HEPCIDIN LEVELS WITH TREATMENT

H. C. Kilinc1, B. O nec2, K. O nec1, E. Caliskan3, H. Ankarali4
1Internal Medicine, 2Hematology, 3Medical Microbiology, 4Biostatistics, Duzce University Faculty of Medicine, Duzce, Turkey

Background: Iron deficiency anaemia (IDA) is a global health problem and problems in compliance with oral iron therapy are frequently seen. It has been shown that medications are not used regularly or discontinued due to weight gain associated with the treatment process.

Aims: We investigated ghrelin, known as appetite hormone and its relationship with hepcidin, the homeostatic regulator of intestinal iron absorption, in order to explain some symptoms of IDA and weight gain during iron treatment.

Methods: A hundred and twenty adult IDA patients, referred to our clinic between October 2015 and October 2016 were included in the study. The study was completed with 87 patients, who gave the informed content and a control group consisted of 50 healthy people. Information about age, gender, weight, height, body mass index (BMI), waist-hip circumference and blood samples were taken from the patient and control groups. The treatment of IDA was done according to the dose and method recommended by the referring physician. The researchers did not have any effect on the treatment. Measurements and blood tests were repeated in the patient group after normalization of the anemia parameters, not before the third month of treatment. Hepcidin and ghrelin levels
Studies should be designed in this regard. With weight gain and change of ghrelin levels. More extensive and controlled.

was significantly lower in the iron deficiency group than in the control group control group in the IDA group, suggesting that it may be the cause of loss of appetite. The deficiency of iron may cause deficiencies in enzymatic activities of iron depended enzymes and it may disturb the function of appetite. Ghrelin is also detected in neurons of hypothalamic arcuat nucleus, consequently responding to hypomethylating agents (Bejar, Lord 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% hematopoietic 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), CDKN2A (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 per 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, BCO1, 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 AZACYTIDINE-TREATED JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML) PATIENTS

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1Orcohaematology, Bambino Gesù Children Hospital, Roma, Italy. 2Pediatrics-Oncohematology, University of Freiburg, Freiburg, Germany

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. Abrupt DNA methylation is a key molecular feature of JMML, suggesting an important role of epigenetic events in the pathophysiology of the disease. Azacytidine (AZA), a molecule that inhibits DNA methylation in human cells, is under investigation in JMML.

Aims: Here we report, for the first time, a global evaluation of DNA methylation status of CD34+ cells deriving from JMML patients before and after AZA treatment and compared the results with those of healthy controls. Identifying differentially methylated CpG islands linked to various genes will help us describe
an epigenetic aberrant paradigm possibly involving transcriptional and translational regulation in JMML.

Methods: CD34+ cells isolated from 3 JMML patients samples collected at diagnosis (t0) and after the third cycle of AZA (t1) were evaluated together with those of 3 healthy donors (HD). JMML patients have been treated with AZA on a compassionate use basis after obtaining signed informed consent. DNA samples were processed and ion fragment libraries were prepared. MBD-seq, bioinformatics and statistical analysis were performed by Genomnia srl (Bresso, Italy).

Results: First, we compared 10 JMML cells with HD cells, finding 987 different transcriptional units corresponding to 714 coding and 273 non-coding sequences. We also compared DNA methylation between t0 and t1. In this comparison, 644 unique transcriptional units, including 468 coding and 176 non-coding sequences, were found. Hypermethylation in JMML samples compared to HD was detected, but, unexpectedly, t0 vs t1 methylation analysis did not show any significant result, suggesting a likely 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-coding elements, we found different RNA species, such as microRNAs, splicing RNAs, lincRNAs/antisense transcripts (AS) and other unknown RNAs. Retrotransposons, belonging to LINEs and SINEs families, were also screened. We identified 13 sequences with a significant differential methylation profile in both t0 and t1 vs HD. Again, a comparison between t0 and t1 groups did not show any significant difference. Eleven hypermethylated common LINEs were evident between t0 vs HD and t1 vs HD sets. Two retrotransposons with opposite methylation patterns were found in t0 vs HD and t1 vs HD sets; while in the first comparison they included LINEs, in the second one they are 1 hypermethylated LINE and 1 hypomethylated SINE.

Figure 1.

Summary/Conclusions: In conclusion, the whole genome MBD-seq performed for the first time on JMML CD34+ bone marrow derived cells, showed a broad genomic hypermethylation both in pre- and post-AZA samples compared to HD, suggesting a patient-specific AZA effect. Transcription and translation processes of coding and non-coding genes could be deregulated in multiple ways, due to heterogeneity of sequences involved in CpG islands hypermethylation. Moreover, due to their known ability to insert random mutations in the genome, retrotransposons should be considered for further studies in JMML pathogenesis.

E1168

RESPONSE MONITORING IN MDS WITH DEL(5Q) USING DIFFERENT FLOW CYTOMETRIC (FCM)-SCORES IN COMPARISON TO CYTOGENETICS AN ELNET IMDS-FLOW EXPERIENCE

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Methods: Overall, 245 FCM investigations were performed in 61 patients with MDS and del(5q) (IPSS-R very low/low: n=26, int: n=13, high/very high n=22) including 42 patients with isolated del(5q) or one additional cytogenetic abnormality. The majority of analyses were performed in patients receiving lenalidomide or azacitidine (n=29 and n=22 patients), or in patients receiving chemotherapy and/or allogeneic transplantation or growth factors (n=10). Standardized FCM (lyse-stain-wash) and cytogenetics/FISH procedures were performed according to ELN guidelines at the TU of Dresden, VUMC of Amsterdam, UH of Guadalajara and UH of Bristol. Cytogenetics/FISH analysis was considered the gold standard. All of the applied FCM-scores were propagated by the ELNet MDS working group. Additionally, hematological improvement of the erythroid lineage (Hi-E) was evaluated (Cheson et al. 2006).

Results: The del(5q)-FCM-score reflected best the disappearance / presence of the cytogenetic abnormality del(5q) with a sensitivity of 82% and a specificity of 86%. This was confirmed if only MDS with del(5q) as a single abnormality or only MDS treated with lenalidomide were evaluated separately (sensitivity: 98% and 100%; specificity: 85% and 75%). The use of the Ogata-score considering almost only abnormalities of the myeloid progenitors, ended up with a slightly lower sensitivity (86%) and specificity (81%). The new iFS analyzing progenitor cells, granulo-, mono-, and erythropoiesis showed a comparably high specificity (83%) but a slightly impaired sensitivity (72%). FCSS, analyzing dyspoiesis of multiple cell lineages, showed a response in less than half of the high sensitivity of 41%, but revealed a higher specificity (91%). The analysis of Hi-E was high sensitive (81%) but not as specific (62%). Next, we investigated the potential prognostic impact of response monitoring using various FCM-scores compared to cytogenetics. Considering all del(5q) MDS patients as well as only those patients with del(5q) as a single abnormality, cytogenetics and all tested FCM-scores showed a significantly longer OS for MDS responding to therapy. The highest prognostic impact distinguished the iFS (p=0.0019) and Ogata-score (p=0.0092), respectively. Evaluating only MDS treated with lenalidomide, response monitoring using FCSS separated best the OS curves (p=0.0080). Finally, we combined the evaluation of Hi-E with cytogenetics or the FCM-scores. This resulted in an even better OS for MDS fulfilling two response criteria with the Hi-E/iFS-criteria as the highest prognostic impact for the combination of Hi-E plus the new iFS (p=0.0010).

Summary/Conclusions: Flow cytometry might serve as a rapid tool for response monitoring during treatment with disease-modifying drugs. All established FCM-scores allowed for an at least similar correctness of response prediction. The prognostic impact of the various FCM-scores seems to be even higher than that of cytogenetic response evaluation in this MDS subgroup. One reason might be, that most of the FCM-scores reflect not only the genetic background of the MDS but dyspoietic alterations in various cell lineages of the hematopoietic system.

E1169

EVALUATION OF MUTATIONS AT RELAPSE IN MYELODYSPLASTIC SYNDROME PATIENTS RECEIVING 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 AlloSCT is a potent prognostic tool. However, mutational profile at relapse after AlloSCT has not been widely explored.

**Aims:** In this study, we evaluate mutational profile at post-AlloSCT relapse in MDS patients to determine if pre-AlloSCT mutations are still present at relapse, so we could eventually monitor them as minimal residual disease (MRD) after AlloSCT.

**Methods:** From a retrospective cohort of 115 patients, we selected those who relapsed post-AlloSCT (19/115, 16.5%) with available material at relapse (18 patients). We performed an in-house target-capture panel, sequencing across selected exons of 117 cancer-related genes previously related to MDS in pre-AlloSCT samples to identify genetic mutations and we checked the presence of those mutations in samples at relapse. Six patients were discarded because of lack of pre-AlloSCT mutations, so we selected 12 patients for the sequential study. DNA was amplified with FastStart High Fidelity PCR System using exon-specific primers for each mutation. The indexed paired-end library was prepared with Nextera XT DNA Sample Preparation Kit (Illumina) The median coverage per base achieved was 4579 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 1 (n=4), RAEB 2 (4), dysplasia associated AML (2) and RCMD (2). They demonstrated a decrease in association of Pol II with H3K4me2 (67%) and in association of H3K4me3/Pol II in BW-90 cells (7-30%; ANOVA, p<0.0001). An overall decrease in association of Pol II with H3K4me2 was observed in the BM from MDS pts (ANOVA, p=0.006), but the RIG/VOR induced 1.9-fold expansion of CD34+ cells in both cell lines. An overall decrease in association of Pol II with H3K4me2 was observed with the combinations (AZA/RIG, VOR/RIG or VOR/VOR) altered DNA methyl transferases (DNMT1, 3a and 3b), the class I, II and IV histone deacetylases (HDACs), and chromatin remodeler (KDM2a, SET1, JMJD3 and LRWD1) transcript levels in a cell line specific context. Sequential treatment of RIG with AZA or VOR demonstrated differential effects. Initial results of an ongoing Phase I/II study with RIG combined with AZA or VOR in sequential treatment (AZA/RIG, RIG/AZA, VOR/RIG or RIG/VOR) altered DNA methyl transferases (DNMT1, 3a and 3b), the cell cycle and histone acetylation (H3K9ac, & H3K18ac) levels associated with transcriptional activation or repression in both the cell lines and pt samples.

**Summary/Conclusions:** 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 pt 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 pt bone marrow samples treated on the phase I/II study, obtained prior to and after one cycle of AZA and RIG. Treatment with RIG alone altered global histone post-translational modifications (PTMs) including methylation (H3K4me3, H3K4me2, H3K27me3, and 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 methyl transferases (DNMT1, 3a and 3b), the class I, II and IV histone deacetylases (HDACs), and chromatin remodeler (KDM2a, SET1, JMJD3 and LRWD1) transcript levels in a cell line specific context. Sequential treatment of RIG with AZA or VOR demonstrated differential effects. Initial results of an ongoing Phase I/II study with RIG combined with AZA or VOR in sequential treatment (AZA/RIG, RIG/AZA, VOR/RIG or VOR/VOR) altered DNA methyl transferases (DNMT1, 3a and 3b), the cell cycle and histone acetylation (H3K9ac, & H3K18ac) levels associated with transcriptional activation or repression in both the cell lines and pt samples.
Madrid, Spain, June 22 – 25, 2017
Results: 156 patients were included between January 2014 and December
2015 with a mean age of 68 years [65.8-70.3] and 47.4% of men. 127 patients
(81.4%) had a bone marrow analysis. 53 patients (34.0%) were reported in the
group “positive NGS” and 103 patients (66.0%) in the group “negative NGS”.
In univariate analysis, significant variable associated with “positive NGS” were
age (p <10-7), no history of auto-immune disease (p=0.002), hemoglobin
<12g/dL (p=0.017), platelets >150000/mm3 (p=0.015), >10% dysplastic cells
in erythroid (p=0.012) and granulocytic lineage (p=0.034). Trend test on dysplastic lineage number was significant (p=0.006). Normal karyotype (78.1%)
was comparable in the two groups (p=0.352). Cirrhosis and/or portal hypertension were comparable in the two groups (14.1%, p=0.092) as well as mean
serum creatinine (p=0.24). In multivariate analysis, age >70 years (p=0.0011)
and platelets >150000/mm3 (p=0.0213) remained significantly associated to
positive NGS (Table 1). In “positive NGS” group, 1 (58.5%), 2 (32.1%), 3 (7.5%)
or 4 (1.9%) mutation(s) were found per patient. Most frequent mutations were
TET2 (25.9%), DNMT3A (17.3%), SF3B1 (12.3%), ASXL1 (12.3%), SRSF2
(8,6%), U2AF1 (4,9%), TP53 (3,7%) and ZRSR2 (3,7%). Other mutations were
reported in less than 3 patients. As expected in this elderly population, if a
unique mutation was found, TET2 and DNMT3A were predominant (35.5% and
25.8% respectively) but interestingly mutation R882 of DNMT3A was found in
only one patient. Sideroblasts were found>15% in 46.2% of patients with a
mutation of SF3B1, SRSF2, U2AF1 or ZRSR2.
Table 1.

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 (SKM1R) to azacitidine, were analyzed for expression of UCK1, UCK2, hENT1,
hCNT3, RRM1 and RRM2 by quantitative PCR. Corresponding proteins were
quantitated by western blotting in both cell lines. The expression of UCK1 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 (OriGene Technologies, MD, USA); cells were cultured at a density of 600x105cells/ml in 5 ml of RPMI 1940 medium. After 72 h
of transfection, cells were treated for further 48h with azacitidine at the concentrations of 0,1 and 1 μM. After assessment of effective gene silencing, apoptosis and cell cycle arrest were evaluated, respectively by Annexin V test and
Propidium Iodide. In parallel, the percentage of 5-methylcytosine was quantitated by ELISA assay (Global DNA Methylation LINE-1 kit ActiveMotif, CA,
USA). In addition, the expression of nucleoside metabolizing enzymes was
evaluated prospectively in 18 IPSS high risk MDS patients treated with azacitidine 75mg/m2/7 days every 28 days. Furthermore, UCK1 and UCK2 expression was evaluated in 37 patients (classified as 26 responder and 29 nonresponder) treated with azacitidine, by RNAseq analysis using DEXseq2.
Results: SKM1-R cells did not express UCK1, UCK2, hENT1, hCNT3, RRM1
and RRM2. Corresponding proteins were also not expressed. A reduction of
apoptosis was observed in UCK1-silenced SKM-1 S after azacitidine 0.1 μM
treatment: 35,7%±0,77% Annexine V-positive cells versus 25%±0,35% (P=0.031)
in non-silenced control SKM1-S cultures. We observed a reduction of apoptosis
during 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 in primary cells did not predict different clinical
response to azacitidine. RNAseq analysis for UCK1 and UCK2 did not find any
differences between responder and non-responder patients.
Summary/Conclusions: We demonstrated that UCK1, UCK2, hENT1, hCNT3,
RRM1 and RRM2 and the corresponding proteins are absent in azacitidineresistant cell line SKM1-R suggesting to be the determinant of the induced
resistance to azacitidine. UCK1 and UCK2 silencing induced by synthetic siRNAs significantly decreased azacitidine effects. Prospective evaluation of the
predictive role of cellular expression of genes involved in azacitidine metabolism
is ongoing in a larger cohort of MDS patients.
E1174

Summary/Conclusions: In the context of unexplained cytopenias, a third of
patients had at least one MDS-associated somatic mutation. Age above 70
years and no thrombopenia seems to be good arguments to realize NGS in
this context. Probably thrombopenia is frequently associated to other causes
than MDS. If NGS is positive, aging genes are the most frequently mutated
genes and they can reflect age-related clonal cytopenias. Even if their clinical
significance is uncertain, monitoring is recommended because of an increased
risk of hematologic cancer.
E1172

Abstract withdrawn.
E1173

RESISTANCE TO AZACITIDINE IS DETERMINED AT CELLULAR LEVEL
BY LOWER EXPRESSION OF NUCLEOSIDE ACTIVATING ENZYMES UCK1
AND UCK2
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Background: Azacitidine is at present the standard treatment for MDS. We
demonstrated that MDS patients responsive to azacitidine have significantly higher intracellular expression of the azacitidine-activating enzyme uridine-cytidine
kinase-1 (UCK1) in bone marrow mononuclear cells (Valencia et al. Leukemia
2014). Correlation of the expression of nucleoside transporter, activating and disactivating enzymes with clinical and in vitro response to hypomethylating drugs
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.

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 telomerase access and
activity (Frank 2015). TINF2 (14q12) is encoding for TIN2, the central component of shelterin 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 TINF2 mutations are known in
Dyskeratosis Congenita (DC) (Savage 2008) and other telomere related phenotypes, i.e. aplastic anemia (AA), idiopathic pulmonary fibrosis, liver cirrhosis,
myelodysplastic syndromes (MDS) and acute myeloid leukemia (Armanios
2012). All mutations were missense and heterozygous, clustering in exon 6
encoding for a highly conserved segment 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 69-year-old man, with a multisystem disorder,
i.e. psoriasis, nail dystrophy, severe osteoporosis, chronic hepatopathy, 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
TINF2 using DHPLC and Sanger sequencing. Q-FISH investigated telomere
length. SNPs were performed following manifacturer’s instructions (Affymetrix).
Paired-end libraries for Whole Exome Sequencing (WES) were generated with
NimbleGen Exome Capture v3 (Roche), according to manufacturer. DNA from
CD3+ population was used for germinal matching. Data were aligned to the
human reference genome (GRCh38/hg38) and analyzed with the in-house
CEQer2 software (Piazza 2013). Mutational analysis and telotype were performed in both proband and familial members. TERF2 and TINF2 coding
sequences were cloned in pGem-Teasy vector and site direct mutagenesis reproduced in vitro the mutation. Using expression vectors, respectively pEGFP-C1
and pDsRed-Express-C1, TRF2 and TIN2 wild type or TRF2 and TIN2 mutated
were co-expressed in HEK-293T cell line. Co-immunoprecipitation was performed with anti-GFP antibody and differences in TRF2 binding between TIN2wt
and TIN2mut were revealed by western blotting.

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Results: A new TIM2F germlinal variation at exon 2, c.254A>G p.H85A, 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 SNPs profile and WES identified an additional somatic variation in TLR1 gene (c. 1859G>A, p.R620Q). Co-immunoprecipitation experiment showed that the new TIM2F mutation reduced TIM2 binding with TRF2 in vitro.

Summary/Conclusions: A new TIM2F germlinal variation at exon 2, c.254A>G p.H85A, 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 SNPs profile and WES identified an additional somatic variation in TLR1 gene (c. 1859G>A, p.R620Q). Co-immunoprecipitation experiment showed that the new TIM2F mutation reduced TIM2 binding with TRF2 in vitro.

E1175

FUNCTIONAL EXPRESSION OF TIM-3 AND CLINICAL SIGNIFICANCE OF PLASMA GALECTIN-9 LEVELS IN MYELODYSPLASTIC SYNDROMES

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Aims: We investigated the expression and function of TIM-3 using a myelodysplastic syndrome (MDS) cell line.

Methods: 1) We evaluated Tim-3 expression on CD45-gating blasts of bone marrow mononuclear cells (MMCs) in 20 patients with MDS and controls (n=22) using FACS sorting. 2) To investigate Tim-3 induction, MMCs were divided into Tim-3+ and Tim-3–fractions with FACS sorting and their differential gene expression was evaluated using oligonucleotide microarray analysis. 3) Intracellular Ki-67 expression in Tim-3+ and Tim-3– Blasts was compared by FACS sorting. 4) To evaluate the proliferative potential of Tim-3– expressing cells, we used MDS cell lines and PBMCs obtained from AL-MDS patients. Soluble Tim-3 was detected in culture supernatants of MDS cell lines and PBMCs obtained from MDS patients. 5) Soluble Tim-3 was detected in culture supernatants of MDS cell lines and in plasma obtained from patients with MDS (n=51) and healthy donors (n=10).

Results: 1) Tim-3 expression was observed on monocytes and CD45-gating blasts in MDS MMCs and in all 4 MDS cell lines. 2) Tim-3 expression levels on blasts were markedly higher in controls and MDS patients with blast counts of ≤10% than those with blast counts >10%. Tim-3+ cells were found in association with the culture supernatant of human stromal cells and MDS-related cytokines. 3) To elucidate the functions of Tim-3 on MDS cells, F-36P cells were divided into Tim-3+ and Tim-3–fractions with FACS sorting and their differential gene expression was evaluated using oligonucleotide microarray analysis. 4) To investigate the proliferative potential of Tim-3– signaling, intracellular Ki-67 expression in F-36P cells was evaluated using FCM when co-cultured with/without anti-Tim-3 blocking antibody. 5) Finally, we analyzed gal-9 concentrations in culture supernatants of MDS cell lines and in plasma obtained from patients with MDS (n=51) and AL-MDS (n=19), and healthy donors (n=10).

Summary/Conclusions: A new TIM2F germlinal variation at exon 2, c.254A>G p.H85A, 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 SNPs profile and WES identified an additional somatic variation in TLR1 gene (c. 1859G>A, p.R620Q). Co-immunoprecipitation experiment showed that the new TIM2F mutation reduced TIM2 binding with TRF2 in vitro.

E1177

SUPPRESSION OF DNA METHYLTRANSFERASE ENZYMES BY A NOVEL HYPMETHYLATING AGENT, SG-1027, IN DECITABINE-RESISTANT CELL LINES

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Aims: To find out clues to overcome the resistance to hypomethylating agent (HMA).

Background: We established azacitidine- and decitabine-resistant cell lines, MOLM-02A and MOLM-DEC-5 from MOLM-13, an acute myeloid leukemia cell line (OncoGenics). DNA methyltransferase (DNMT) 3B was upregulated in the resistant cell lines.

Methods: Single nucleotide polymorphism (SNP) arrays (Tu et al., Blood, 2012) have shown a potential for score criteria in the diagnosis of MDS. Recent reports of the presence such genetic aberrations in disease free individuals makes this approach problematic (Genoves 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 (Canto et al., Blood. 2015). The presence of mutations with greater median variant allele fraction (40% vs 9% to 10% in healthy individuals) and occurring with additional mutations (>2 mutations, 64% vs 8%) were shown to define a high-risk group with a shorter time to disease progression and poorer overall survival.

Aims: To compare the genomic profile of bone marrow from 145 adults, 76 of whom met the WHO criteria for MDS.

Methods: All samples were screened by chromosome G banding or molecular karyotyping using 8x60K oligonucleotide arrays (Agilent, USA) or screened by FISH using probes (Cytocell, UK) targeting the most common aberrations associated with MDS as per IPSS-R classification (Greenberg et al., Blood, 2013). The commercially available target gene panel TruSight on a MiSeq platform (Illumina, USA) was used to screen mutational hotspots in 5 cancer-related genes relevant to myeloid malignancy. Genomic mutations were reported at allele frequencies (VAF) ≥1% 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 variant genes as either drivers, variants of unknown significance and germline polymorphisms (SNPs).

Results: A total of 145 bone marrow samples from 58 women and 87 men, aged from 26 to 85 suspected to have myeloid dysplasia were investigated. Of these only 76 (52%) were found to fulfill the WHO criteria referred to as MDS positive, the rest as MDS negative. Genomic variants were detected in all but 7 samples. The latter were considered void of gene mutations. We observed driver mutations as reported in myelodysplasias in 68 (47%) samples whilst 70 (48%) were found to carry the same variants seen in disease free individuals or of unknown significance. As expected driver variants were not identified in any of the samples that failed the WHO criteria for MDS. Variants were detected in all samples for 35 of the 54 genes targeted by the TruSight myeloid panel. In order of frequency these are TET2, SRSF2, ASXL1, CUX1, DNMT3A, RUNX1, BCROR1 and HRA5, seen in more that 10% of all samples, while the rest were less frequently reported. The aberrant genes ASXL1, TET2 and SRSF2 that were commonly found in both groups of samples with comparable TRUSIGHT allele frequency (>0.1) when in contrasted with the absence of aberrant genes unique to the MDS group albeit at low frequency. When we examined the distribution of individual variations (rather than genes) we found a unique 1oci of the genes ASXL1, TET2 and SRSF2 is 101922441, U2AF at 2614361248 and TET2 at 101927025 to be associated with the MDS positive group. A more detailed analysis on the significance of these findings will be presented.

Summary/Conclusions: We compared 145 bone marrow samples from patents 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 occurrence of the most highly involved genes (ASXL1, TET2 and SRSF2) but we compare the two groups by variance, 9 variances are exclusively associated with MDS positive disease.
Methods: Besides azacitidine and decitabine, three other agents (SGI-1027, zebularine, and gemcitabine) are known as having hypomethylating effect. In vitro activities of the 5 HMA's on HMA resistant cell lines (MOLM/AZA-1 and MOLM/DEC-5) were tested by cell viability assay using luminescent-based CellTiter-Glo system. Protein and mRNA levels of DNMT enzymes (1, 3A, and 3B) were assayed before and after treatment of each HMA. Protopoiesis degradation and activation of Akt were also determined to see the correlation with changes of DNMT's.

Results: Although azacitidine and decitabine could suppress DNMT1 and DNMT3A in MOLM-13, the agents could not suppress DNMT3 enzymes in resistant cell lines. Inhibition of protopoeisal degradation by bortezomib induced accumulation of DNMT enzymes in MOLM-13, whereas it did not accumulate the enzymes in MOLM/AZA-1 and MOLM/DEC-5. Phosphorylated Akt (p-Akt) was dramatically overexpressed in MOLM/AZA-1 and MOLM/DEC-5, SGI-1027 showed the lowest IC50 values for MOLM/AZA-1 and MOLM/DEC-5, and it suppressed the protein levels of all three DNMT enzymes. SGI-1027 could also decrease the level of p-Akt. GDC-0941, a PI3K inhibitor, suppressed DNMT1 and DNMT3A as well as p-Akt, but it could not decrease DNMT3B in MOLM/AZA-1 and MOLM/DEC-5. Cell viability assay showed the synergistic effects of combination of GDC-0941 and 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 protopoeisal 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: Deciper 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 NFkB detected by both CM (p=0.04) and luciferase reporter assay (p=0.03). NFkB 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 NFkB activation. These results suggest that NFkB activation in this model is linked to TRX1 and regulated by an extremely fine control of ROS levels with a likely threshold effect.

Summary/Conclusions: Our study describes the pro-proliferative effects of low dose of DFX on erythroid progenitors in low risk MDS patients. These results provide a biological rationale for a clinical trial which will propose low dose of DFX in MDS patients, refractory to erythropoiesis stimulating agents.
Myelodysplastic syndromes – Clinical

E1179
EVALUATING ERYTHROBLAST PAS POSITIVITY IN THE DIAGNOSTIC APPROACH OF MYELODYSPLASTIC SYNDROME
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Background: According to WHO minimal morphological criteria for myelodysplastic syndrome (MDS) diagnosis, at least 10% of bone marrow (BM) cells of at least one hematopoietic lineage must show unequivocal dysplasia to be considered as dysplastic. Morphological abnormalities of erythroid cells include cytoplasmic Periodic acid-Schiff (PAS) positivity, but the diagnostic power of this cytochemical reaction is not yet fully clear.

Aims: The aims of our study were to evaluate the diagnostic significance of erythroblast PAS positivity in MDS and to investigate a possible correlation between levels of PAS positivity and other morphological and clinical features.

Methods: We retrospectively examined the results of the cytochemical PAS staining for glycogen in BM smears from 165 patients with MDS, 116 patients with non-clonal cytopenia and 49 healthy subjects. We developed a PAS score by counting 100 nucleated cells for the erythroid lineage and classifying them according to the degree of PAS reactivity. The discriminant power of both PAS positivity rate and score for MDS identification was evaluated in comparison with that of the conventional morphological features of dyserythropoiesis; then, PAS positivity was included into the morphological scoring system we have previously defined (Leukemia 2015;29:66-75).

Results: PAS positive erythroblasts were observed in 104 (63%) MDS patients, 46 (40%) patients with non-clonal cytopenia, and 12 (24%) non-erythroid controls, with a significant difference between MDS and non cytopenic controls (p<0.001) or non-clonal cytopenias (p=0.001), 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 difference in relation to excess blasts or multilineage dysplasia. MDS patients with >4% ring sideroblasts (RS) showed lower PAS positivity rates and scores than MDS patients with ≤4% RS (p=0.0332 and p=0.0412, respectively). In MDS-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 noticed between PAS score values and intercellular bridging (r=-0.23, p=0.0395). A ROC curve analysis allowed us to identify a PAS score value ≥1 (AUC=0.674, p=0.0034) as optimal cutoff to discriminate MDS patients from non-clonal cytopenias. Considering the most discriminant morphological features for dyserythropoiesis, the weight of both PAS positivity rate and score in the identification of BM dysplasia was lower than that of ring sideroblasts and megaloblastosis, but higher than that of defective hemoglobinisation, nuclear lobulation, multinuclearity, cytoplasmic fraying, pyknosis, and internuclear bridging. Introducing conventional parameters and PAS results significantly improved the specificity 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 unilateral 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 MYELODYSPLASTIC SYNDROMES (MDS)
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Background: To evaluate darbepoetin alfa (DAR) in IPSS low/int-1 risk MDS patients, 24 wk of darbopeotin alfa Q3W significantly reduced transfusions and increased HI-E rates with no new safety signals. Most patients met criteria to change to Q2W dosing during the 48-wk OL period, suggesting that Q2W dosing may offer more benefit. The true clinical benefit of darbopeotin alfa may have been underestimated due to the nature of IWG 2006 HI-E criteria and trial design (Hb measured Q3W, dosing rules).

Methods: Patients with MDS per WHO 2008 criteria with IPSS low/int-1 risk, anemia [hemoglobin (Hb)≤10 g/dL], low transfusion burden, no previous treatment with ESAs or biologic response modifiers, and serum EPO<500 mIU/mL were randomized 2:1 to 24 weeks (wk) SC DAR 500 μg or PBO every 3 wk (Q3W), stratified by IPSS, then 48 wk open label (OL) DAR; follow-up is ongoing. Doses were withheld for Hb>12g/dL and decreased if Hb increased by >1.5 g/dL in 3 wk. Key endpoints were transfusion incidence and HI-E per IWG 2006.

Results: Randomized patients [N=147] had median Hb of 9.3 (min-max:5.5-10.6) g/dL and median baseline EPO of 69 (min-max:4.3-497) mU/mL. WHO classification was RA-RS in 21%, RARS in 14%, RARSi in 4%, del(5q) in 9%, RAEB-1 in 16%, RAEB in 2%, and MDS-U/unknown:2%. Transfusion incidence wk 5-24 was significantly reduced with DAR [DAR:36.1% vs PBO:59.2%, p=0.008]. In the 48-wk OL DAR period, 50.8% of patients had transfusions. More DAR patients achieved HI-E in the double blind period [DAR:14.7% (11/75 evaluable) vs PBO:0% (0/35 evaluable), p=0.016]. In the 48-wk OL DAR period, 34.7% (34/88) of patients achieved HI-E. Improved HI-E and transfusion responses were seen with more favorable status for IPSS-R but not IPSS. In the 48-wk OL DAR period, dose frequency increased from Q3W to Q2W in 81% of patients; doses were held/reduced frequently. Safety results from this trial were consistent with the previous DAR phase 2 MDS trial, with similar AML rates in PBO and DAR arms.

Figure 1.

Summary/Conclusions: In this phase 3, randomized, double-blind, PBO-controlled trial in anemic IPSS low/int-1 risk MDS patients, 24 wk of darboptoeitin alfa Q3W significantly reduced transfusions and increased HI-E rates with no new safety signals. Most patients met criteria to change to Q2W dosing during the 48-wk OL period, suggesting that Q2W dosing may offer more benefit. The true clinical benefit of darboptoeitin alfa may have been underestimated due to the nature of IWG 2006 HI-E criteria and trial design (Hb measured Q3W, dosing rules).
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 Sistran-Rev trial, a phase III, multicenter, randomized and double-blind study with LEN (5mg/day) vs placebo [2:1 randomization] in Low – Int-I risk (IPSS) MDS del(5q) patients with anaemia but TI. Preliminary results of efficacy (according to the IWG 2006 criteria for erythroid [Hi-ER] and cytogenetic response [CyR]) and safety have been analyzed at week 12. Progression disease (DP) in the trial was defined as the development of TD.

Table 1.

<table>
<thead>
<tr>
<th>Age median</th>
<th>37-39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M/F</td>
<td>7/40</td>
</tr>
<tr>
<td>Platelets</td>
<td>263 ± 104 (10E12/L)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.18 ± 0.69 (10E9/L)</td>
</tr>
<tr>
<td>Hb (g/dL) median</td>
<td>11</td>
</tr>
<tr>
<td>IPSS-R (n %)</td>
<td>Low very-low</td>
</tr>
<tr>
<td>Very low</td>
<td>15 (31.9)</td>
</tr>
<tr>
<td>Low</td>
<td>26 (55.3)</td>
</tr>
<tr>
<td>Inter</td>
<td>8 (17.0)</td>
</tr>
<tr>
<td>High</td>
<td>2 (4.2)</td>
</tr>
<tr>
<td>Very high</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Neutrophils in BM (%)</td>
<td>39 (83)</td>
</tr>
<tr>
<td>Neutrophils del(5q)</td>
<td>42 (88)</td>
</tr>
<tr>
<td>Neutrophils del(5q) + other del</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Neutrophils Unk</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Neutrophils not eval</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

Results: Main clinical characteristics are summarized in table 1, 85% were females, median age was 72 years (37-89) and most of patients (95%) had del(5q) as the only cytogenetic abnormality. Among 47 patients, only 38 were evaluable at week 12 (6 out of 38 discontinued the study; 3 due to DP, 1 due to toxicity and 1 for unknown reasons), 7 patients are currently receiving the first 12 weeks of treatment and 2 patients were excluded (screening failures). Regarding efficacy (w12), data from 36 patients were available. Hi-ER was observed in 14/36 patients (39%), minor Hi-ER (Hb increased<1.5g/dL) in 4/36 (11%), stable disease in 15/36 (42%) and PD (transfusion dependency) in 3 (8%). CyR was available in 30 patients: complete CyR was obtained in 12 (40%), partial CyR in 6 (20%) and no CyR in 12 (40%) patients. Safety information in 38 patients demonstrated that most patients (87%) developed any adverse events (AE) while only 42% of these were relevant (G3-4). Most G3-4 AE were hematological (neutropenia 38%) being non-hematological only in 5%. Seven serious AE were reported in 5 patients: vestibular neuritis, congestion, pericardial effusion, pulmonary infection and chronic obstructive pulmonary disease exacerbation. All SAE were not related with the drug of the study (LEN/Placebo).

Summary/Conclusions: In this study we confirm a high rate of erythroid and cytogenetic responses early after treatment with an adequate safety profile in the first 12 weeks of treatment with LEN or placebo.

E1182

MYELODYSPLASIA-RELATED MORTALITY REMAINS THE MAIN CAUSE OF DEATH ALONG DIFFERENT GROUPS OF RISKS: AN ANALYSIS FROM MDS ARGENTINEAN STUDY GROUP

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Background: Myelodysplastic syndrome (MDS) are the most frequent hematological malignancy in elderly patients. The impact of MDS burden over overall mortality remains controversial, moreover, after the incorporation of hypometabolizing agents in the therapeutic armamentarium.

Aims: We aimed to analyze overall mortality and causes of death in our population of patients with MDS.

Methods: A retrospective analysis of patients with MDS reported to Argentinean MDS registry and a previous study from Academia Nacional de Medicina. Causes of death were classified in: acute myeloid leukemia (AML), infections, bleeding, solid tumor, cardiovascular, transplant related mortality (MRT), others and unknown. AML, infections and bleeding were considered as MDS-related mortality. Causes of death were analyzed using cumulative competitive event 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) and most being males (53%). 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 years of diagnosis (p=0.291) neither according to age group. Multivariate analysis for cumulative incidence of overall mortality found Charlson index (HR 1.38; p<0.001), sex (HR 1.45; p=0.014) and IPSS-R (HR 2.79; p=0.001) as prognostic variables. The main cause of death was AML accounting for 9% at 12 months (95%CI 7-11), 16% at 24 months (14-19) and 25% at 60 months (95%CI 22-28) of mortality 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 autologous transplant; cumulative incidence of MRT for all cohort was 0.5% at 12 months (95%CI 0.2-1.2) and 1.4% at 24 months (95%CI 0.8-2.4). Only male sex was associated with a higher cumulative incidence of mortality by solid tumor (p=0.001) and a Charlson index ≥2 was associated with higher cumulative incidence of cardiovascular mortality (p=0.021).

Summary/Conclusions: In this large cohort of patient with MDS we demonstrate that MDS-related causes are the leading cause of death along all IPSS-R groups. The absence of difference in mortality along the years of diagnosis highlights the necessity of better treatments for these patients.

E1183

PROSPECTIVE STUDY OF FLOW CYTOMETRY OF BONE MARROW IN 105 CONSECUTIVE PATIENTS WITH CYTOPENIA AND SUSPICION OF MYELODYSPLASTIC 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 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 of myelodysplasia in elderly patients.

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 diagnostic 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-, +8, 20q- and del(7q) cytogenetic abnormalities were performed. 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), del5q Syndrome (3) and Unclassified (2). 4 pts being diagnosed of CML. MFC score was MDS-suggestive in 56 cases, MDS-notch suggestive (36) and in 13 cases its use was precluded because of morphology findings. Considering cytomorphology as gold standard, MDS was diagnosed in patients with MDS-suspected but MFC score was MDS-confirmed (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 a 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 score >2 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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Background: Therapy for patients with HR-MDS includes systemic chemotherapy, stem cell transplant (SCT), and supportive care aimed at improving symptoms associated with MDS-related disruption of normal hematopoiesis. However, the economic impact of these interventions over time for HR-MDS patients has not been fully examined. Aims: We evaluated the costs and healthcare utilization (HCU) of US HR-MDS patients treated during routine care. Methods: Newly diagnosed adult HR-MDS patients who initiated first-line therapy were identified from Optum, a large US claims database, between 1/1/08 and 10/31/15. HR status was based on ICD coding: ≥1 inpatient claim or ≥2 outpatient claims with ≥1 HR-MDS (ICD-9 code: 284.7; 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, erythrocyte- and colony-stimulating- growth factors and erythrocyte/platelet transfusions) and pharmacy claims for MDS treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPM) costs adjusted to 2015 US dollars and reported as mean (standard deviation [SD]). Patients were a capped payment plan were excluded from the cost analysis. Patients were followed until death, propension score matching or end of study (12/31/2015). Results: 209 treated HR-MDS patients were identified. During the follow-up period, 69.4% of patients had ≥1 inpatient admission, but only 8% of patients had an MDS-related than non-MDS-related admission (Table 1). 56.9% of patients had ≥1 MDS-related and 1 outpatient claim, 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, erythrocyte- and colony-stimulating- growth factors and erythrocyte/platelet transfusions) and pharmacy claims for MDS treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPM) costs adjusted to 2015 US dollars and reported as mean (standard deviation [SD]). Patients were a capped payment plan were excluded from the cost analysis. Patients were followed until death, propension score matching or end of study (12/31/2015). Results: 209 treated HR-MDS patients were identified. During the follow-up period, 69.4% of patients had ≥1 inpatient admission, but only 8% of patients had an MDS-related than non-MDS-related admission (Table 1). 56.9% of patients had ≥1 MDS-related and 1 outpatient claim, and non-MDS-related HCU and costs incurred during follow-up were evaluated. The majority of patients had ≥1 physician office visit (91.9%) and other outpatient visits (99.5%). Over the follow-up period, the mean PPPM 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 differences in 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.

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 the 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. Hematoblogical response was assessed by IVG criteria 2006. Hemolysis response (HLR) included normalization (CR) or a greater than 50% improvement (PR) of LDH, reticulocytes, indirect bilirubin and haptoglobin.

Results: Clinical characteristics are shown in the Table. All patients had a chronic cytopenia and 17 patients (94%) had a monoclonal T-cell population over the follow-up period. In 9 cases the clone was characterized by flow cytometry: 6 had a CD3+ T-cell and 3 had a CD3-/CD16+/CD56+ NK-cell expansion. Associated immunologic disorders were: ITP (4), neutrophil dermatosis (3), inflammatory bowel disease
(3), seronegative arthritis (2), connectivitis (2). One patient with hypoplastic MDS had LGL liver involvement. Coombs test was positive in 4/16 hemorrhage 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) to 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 C3 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 make this drug a valuable option not only in LR but also in HR patients, although a confirmation on larger cohorts is needed. IVIG at intermediate-high dose suppresses proliferation of T-cells and induces immune-regulation. Given the relative rarity of T-cell clones in MDS, further investigational studies are underway to define their pathogenetic role and the mechanism of action of IVIG in this specific subset of patients.

E1186
DEVELOPMENT AND EXTERNAL VALIDATION OF A NEW PATIENT-CENTERED PROGNOSTIC INDEX FOR PATIENTS WITH ADVANCED MEYLODYSPLASTIC SYNDROMES
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Background: The clinical presentation of myelodysplastic syndromes (MDS) is highly variable, and the ability to accurately predict outcomes is critical. Current prognostic systems for these diseases are based on traditional clinical, pathologic and laboratory indicators.

Aims: We aimed to develop and validate a new prognostic index for advanced MDS by including self-reported fatigue severity into a well-established clinical risk classification: the International Prognostic Scoring System (IPSS).

Methods: Untreated patients (n=280) were recruited at the time of diagnosis of advanced MDS from 37 hospitals in nine countries to create the index. The index was then applied to an independent cohort including pre-treated MDS patients from the Dana-Farber Cancer Institute (DFCI) in Boston, Massachusetts (USA: n=189). Patients in both the international and DFCI cohorts were adults with newly-diagnosed intermediate-2 or high-risk MDS (advanced disease on the IPSS). Patients were enrolled regardless of age, comorbidity, performance status and progression. The index yielded high rates of durable response on all lineages and on hemolysis. Trans-dose suppression of proliferation of T-cells and induces immune-regulation. Further investigation 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).

Summary/Conclusions: The IPASS-10 is an additional prognostic tool that might enhance clinicians’ ability to provide more personalized treatment strategies both in untreated and pretreated advanced MDS patients. This analysis offers a model for integration of PROs in prognostic systems for patients with other cancers and advanced illnesses.
Results: Overall, no significant correlation was seen between expression of p53 and degree of fibrosis (p>0.25). However, degree of fibrosis predicted for overall survival in patients with p53 expression (median overall survival of 4 months in patients with both p53 over expression and significant fibrosis compared with median overall survival of 18 months in patients with p53 over expression without fibrosis, p<0.001). In patients who received azacitadine, though the number of significant fibrosis patients and positive for del(5q) was small, an increased overall survival compared with those who did not receive azacitadine (4 months versus 1 month, p=0.002). Azacitadine treatment was not associated with increased survival in patients with p53 expression without fibrosis but these patients did have an overall increased survival compared to those with fibrosis. Since median survival 12 vs 37 months.

Summary/Conclusions: This study confirms that significant marrow fibrosis adversely affects overall survival in patients with MDS, including those with p53 over expression. Patients who received azacitadine had a significant increase in median survival. Although the numbers of patients who received azacitadine were small, this data suggests that patients with fibrosis may benefit from the use of azacitadine and larger and randomized studies should be considered to study this further.

References

E1189
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 most commonly used probes include the search for monosomy 5 or del(5q), monosomy 7 or del(7q), del(20q) and trisomy 8 (T8). In our lab, FISH was historically performed on the whole unsorted patient sample (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 2017 in which a FISH panel was performed due to a suspicion of myelodysplasia, using probes for del(5q), del(7q), del(20q) and T8. The proportion of patients positive for the test, as well as the proportion of positive cells within a positive sample, were compared.

Results: We obtained valid results for 328 samples during the relevant time-frame. 39.6% of which were collected from female patients. FISH was performed after FACS in one third of samples (35.1%, n=115), starting in 2015. Considering the overall cohort, nearly a quarter of samples (23.8%) had at least one aberration in the four probes tested in this study. This proportion of aberrations was significantly higher in double in FACS compared to Full Sample (8.5%): FACS patients (33.0%) compared to full sample patients (18.8%, p=0.004). Del(5q) was present in 5.6% of the cohort; however, positivity was 8-fold higher in FACS patients, compared to full sample patients (12.3% vs 1.6%, p<0.001). Considering the percentage of positive cells in each sample, it doubled from 38.7±29.9% in the full sample to 71.8±28.1% after FACS, p=0.08. Del(7q) was similarly present in 5.7% of the cohort; however, in contrast, there were no relevant differences between FACS patients, 4.2% of whom had del(7q), and full sample patients (8.1%, p=NS).

There were, however, differences in the percentage of positive cells within the sample, doubling from 32.1±11.2% in the full sample to 77.6±17.8% after FACS, p<0.001. Del(20q) was similarly present in 5.7% of the overall tested cohort; the asymmetry in results was marked, with a 36-fold higher proportion of positive findings after FACS (18.7%) compared to full samples (0.5%, p<0.001). The percentage of positive cells doubled from 15% in the single positive test in the full sample cohort, to an average of 35.5±22.2% after FACS. Finally, T8 was found in 10.1% of FACS samples, which was unchanged compared to both full and FACS samples, p=NS.

Summary/Conclusions: We found that one quarter of all patients who underwent a FISH panel workup for a suspected diagnosis of MDS presented with aberrations in at least one of the four selected probes, a proportion which was significantly lower (one fifth) when a full sample was analyzed, and significantly higher (one third) in FACS purified blast cells. Although the purification of the sample through FACS doubled the percentage of positive cells within each sample for all four probes, the likelihood of obtaining a positive result for del(7q) and T8 in the cohort was unaffected by the methodology used. In contrast, the use of a sorted sample greatly increased the proportion of positive findings in del(5q) and, especially in del(20q), the two probes for which the basal positivity in full samples was lowest. The clinical value of this increased rate of detection of del(5q) and del(20q) remains unclear, since their prognostic utility has only been established in levels detectable by conventional karyotyping of a full sample.

E1190
SUCCESSFUL TREATMENT WITH DANAZOL FOR MYELODYSPLASTIC SYNDROMES AND APLASTIC ANEMIA REFRACTORY OR INELIGIBLE TO STANDARD THERAPY
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Background: The discovery of danazol potential activity on telomere elongation in bone marrow failure has renewed interest in this drug. The treatment of cytopenia in myelodysplastic syndromes (MDS) and aplastic anemia (AA) patients who fail or are ineligible to standard therapies is an unmet medical need; however only dated reports on danazol use in this setting are available.

Aims: We report the results of treatment with danazol in patients with MDS and AA at a single institution.

Methods: From Jun–11 to May–15, danazol was administered to 31 consecutive patients (20 MDS and 11 AA). Criteria for treatment were non-serious AA (8), severe AA ineligible/refractory to immunosuppressive therapy or allogeneic transplantation (3), transfusion dependent (TD) lower risk MDS refractory to red cell transfusions (5), 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 assessed by IWG 2006 criteria.

Results: The characteristics of the patients are shown in the Table. All MDS patients had low-risk disease according to IPSS and IPSS-R, except 2 and 3 patients respectively. Nineteen patients (12 MDS, 7 AA) received danazol at full dose (600mg daily). A 400mg daily dose was given to 12 patients, due to toxicity (4 MDS, 4 AA) or comorbidities (4 MDS). Median duration of treatment
was 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), hypoxia (1 G1). Responders to danazol had a better survival in terms of OS and EFS in both groups (Figure 1).

Table 1.

| PFS from initiation of 1LT | Overall | EFS
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| 12 mo                     | 73%     | 50%
| 24 mo                     | 59%     | 38%
| 36 mo                     | 51%     | 31%
| 48 mo                     | 51%     | 31%

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 anaemia 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 anaemia by using dana- zol 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 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: E58.83). 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 hour intravenous decitabine infusion (IV-DAC) in a dose-escalation phase 1 study. (Garcia-Manero. Blood 2016 128:112)

Methods: Adult patients with Int-1/int-2 or HR MDS or Chronic Myelomonocytic Leukemia (CMML) were enrolled in a randomized cross-over Phase 2 study. Patients were randomized 1:1 to receive in the first 28 day cycle, either 5 days of IV-DAC or 5 days of ASTX727, followed by a cross-over to the other in Cycle
2. Cycles 3 Forward were with ASTX727. PD were assessed with LINE-1 methylation as measured on bone cells at baseline and days 8, 15, 21 and 28 in cycles 1 and 2. Full PK assessments of ASTX727 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 ASTX727 and IV-DAC was created for each patient. Safety and clinical response were assessed on all patients.

Results: 50 patients were randomized, 46 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 ASTX727 and IV-DAC were performed independent of sequence. The geometric mean maximum demethylation was 9.9% for ASTX727 and 7.1% for IV-DAC [Ratio of geometric mean AUC of IV-DAC vs ASTX727 = 1.21, 90% CI: 0.80-1.85] with a lower limit of 90% CI of 0.804. The geometric mean AUC for IV-DAC was 161 ng*h/mL. The 5 day total geometric mean of the AUC (ng*h/L) was 769 for ASTX727 and 805 for IV-DAC [(Ratio of geometric mean of ASTX727 and IV-DAC = 0.94, with a lower limit of 90% CI of 0.806). Demethylation Cmax was higher for IV-DAC (189 ng/ml) than after ASTX727 (295 ng/ml) and 5 h (129 ng/ml). The Day 5 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 ASTX727 regardless of relationship to treatment.

Summary/Conclusions: Patients treated with azacitidine (especially APL patients) at high risk of infection were further analyzed to determine if AZA as a monotherapy was associated with a lower infection risk. No further stratification analysis was done. Based on the analysis, the use of AZA in patients at high risk of infection was not independently associated with a lower infection risk. Further stratification analysis is needed to identify if AZA is associated with a lower infection risk in any specific patient population or subgroup.
suitable, but, at present, its safety is questioned in MDS patients. Furthermore, in clinical practice, danazol, an attenuated androgen, has been reported to have some ability to increase the platelet count in this context (Wattel 1994; Chan 2002).

Aims: To assess efficacy and toxicity of danazol employed to improve severe thrombocytopenia in lower-risk MDS setting.

Methods: We retrospectively reviewed twenty-four patients affected by MDS and treated with danazol for thrombocytopenia. The initial dose was 600mg/day for all patients. The IWG criteria of response (Cheson 2006) were adopted. The outcome was observed every 3 months till 12th month. The overall response rate and the average platelet count or each time of observation were described. Progression free survival was estimated with the Kaplan-Meier product limit method, followed by the logrank test and by the Cox proportional-hazard regression.

Results: Of the 24 patients, 3 patients had a therapy-related MDS. At the starting time of danazol therapy, the IPSS was “low” in 9, “int-1” in 13 and “int-2” in 2 cases respectively; the IPSS-R was “very low” in 2, “low” in 11, “intermediate” in 7 and “high” or “very high” in 4 cases. At baseline in 14 patients the platelet count was lower than 20x10^3/mL, the average was 20x10^3/mL and the maximum value was 38x10^3/mL. The median dose was 600mg (range 200-600) also maintained at least up to 3 months (range 400-600). At 6 and 12 months the median dose therapy was 400mg (range 400-600 and 200-600 respectively). The response rate was 79.1% (19 responders on 24 treated). The average count increased as shown in Figure 1, over 60x10^3/mL after 6 months from the beginning of therapy and so maintained after one year. Only 3 patients lost the response at 187, 600 and 633 days respectively. The median survival was not reached in the presented series, and the probability to maintain the response is over 75% after two years from the beginning therapy in the responder patients (Figure 2). Adverse events recorded were as follows: moderate (grade 3) (with subsequently drug suspension); severe (grade 3) but reversible renal failure in 1 case (the drug was stopped); moderate (grade 1 and 2) increase 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 woman.

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

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Background: Treatment of patients with HR-MDS includes hypomethylating agents (HMAs) (azacitidine and decitabine), high-intensity induction chemotherapy (IC), and stem cell transplant (SCT). Given the rarity of disease, information available on how these treatments are applied in practice is limited.

Aims: We evaluated the treatment patterns of HR-MDS patients engaged in routine care within the US.

Methods: Newly diagnosed HR-MDS patients who were ≥18 years old were retrospectively identified from Optum, a large US claims database between 1/1/2008 and 10/31/2015. HR status was based on ICD coding: ≥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)1 initiated on or after the index date. Patients were followed until death, end of continuous enrollment, or end of study (12/31/2015). For patients with progression to acute myeloid leukemia (AML), treatment pattern evaluation stopped at AML diagnosis.

Results: 335 newly diagnosed HR-MDS patients were identified; 209 (62.4%) were treated with 1LT with treatment initiated within 1 month of diagnosis (median: 17 days, interquartile range [IQR]: 9, 35). A higher proportion of untreated patients (n=126) was ≥75 years of age (71.4% vs 61.5%) 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=184), followed by decitabine in 20.6% of patients (n=43), and immunomodulators (lenalidomide or thalidomide) in 8.1% of patients (n=19) (Figure 1). A patients had only SCT and an additional 14 had SCT at some point during follow-up. With regard to HMA therapy, median duration was 4.5 months (IQR: 2.6, 9.5) for azacitidine and 4.8 months (IQR: 2.1, 11.6) for decitabine. A greater proportion of decitabine-treated patients...
received supportive care with colony-stimulating factors (CSFs) (39.5% vs 28.5%) and either erythrocyte or platelet transfusions (69.8% vs 57.6%) during 1LT vs azacitidine-treated patients. Second-line therapy (2LT) was administered to 30 (14.4%) patients; the HMAs again predominated in 63.3% of patients (n=19). Of patients not receiving 2LT, 65 (31.7%) progressed to AML; 47 (22.9%) had <30 days of follow-up due to proximity to end of study (38 [80.9%] of these were on 1LT at end of study data). 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 (42,322bp) was analyzed. Validation was performed by re-sequencing on the Roche 454. In all cases the same target region consisting of 19 genes on Illumina HiSeq. The second test set covered 89 MDS patients, sequenced to train our pipeline, we analyzed two data sets covering data of 54 myelodys-

Methods: We developed appreci8, a variant calling pipeline combining the output of eight open-source variant calling tools: GATK HaplotypeCaller, Platypus, VarScan, LoFreq, FreeBayes, SVNver, SAMtools 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 consider variant calling results in clinical routine, it consistently features lowest PPV as well (set 1: 0.99, set 2: 0.94). The PPV of the appreci8 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 sensitivity 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 appreci8 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 appreci8 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 appreci8 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 treat- ment 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 azacitidine (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 groups in 2 years estimated overall survival (OS), (42% for SCT vs 36% for HMA), a sig- nificant 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 start- ing the treatment, 2 patients treated with HMA (5.3%) and 9 patients treated patients (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.

Reference:
Czech Republic

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 pharmaco-kinetics 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 dys-functions. 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 admin-istration term, followed by 7-day monitoring. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results, 2) efficacy as assessed with the International Working Group 2006 criteria, and 3) pharmacokinetics.

Results: Between March 2013 and November 2014, 6 male and 3 female pts (median age: 70; range 52-80) were enrolled. ECOG PS was 0 in 7 pts and was 1 in 2 pts, and 3 and 6 pts were eventually assigned to the 280 and 560mg BID arms, respectively. According to the FAB classification, 4, 2, 2, and 1 pts were categorized to RAEB, RARS, RA, and RAEB-1, respectively. The prog-nostic 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 dia-betes 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 a urinary tract infection. Six pts developed 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 abnor-malities—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-; 1/9 pts). Among the PK parameters, inter-individual variability was observed in the Cmax and AUC. However, changes suggesting the accumulation of rigosertib during repeated oral administration (e.g., consistent increases in the Cmax and AUC) were not found.

Summary/Conclusions: The present chemotherapeutic 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.

Myeloma and other monoclonal gammapathies - Biology

NON-OVERLAPPING PROMOTER AND SUPERENHANCER DRIVEN PROCESSES SUPPORT MYELOMA CELL GROWTH AND SURVIVAL VIA DISTINCT REGULATORY AXES

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Background: We have previously reported that E2F1 and its heterodimerization partner DP1 promote MM tumor proliferation both in vitro and in vivo; and observed an inverse correlation between their expression and patient survival suggesting a role in MM pathogenesis. Moreover, 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 was to defined the regulatory landscape of E2F in MM to better understand how E2F1 and DP1 drive myeloma cell proliferation; and to define the relationship between promoter proximal transcription factor-associated gene expression and super-enhancer-driven transcriptional programs.

Methods: We integrated genetic perturbation with functional omics to define E2F role in MM. Global occupancy of E2F1 and DP1 in MM was evaluated by Chip-seq analysis. E2F1 and DP1 genomic localizations were then integrated to MM reference epigenome. Enhancers and super-enhancers were mapped using Roadmap data and calculated using bamliquidator (github.com/bradnerlab/pipeline). Read densities were calculated using bamliquidator (github.com/bradnerlab/pipeline/wiki/bamliquidator).

Results: Integration of E2F1 and DP1 genomic localization to MM reference epigenome revealed specific co-occupancy of the factors at promoters of active genes marked by H3K4me3, with a strong positive correlation between E2F and RNA PolII occupancy at H3K4me3 peaks. Road map peaks were marked by H3K27ac and BRD4, showed virtually no E2F binding. Prompt by these observations, we explored the transcriptional and functional interrela-relationship between E2F and BETs to identify their individual contribution to event-ual functional effect in MM. Unbiased hierarchical clustering revealed distinct regulatory axes for E2F and BETs, with E2F predominantly localized to active gene promoters of growth/proliferation genes and BETs disproportionately at enhancer- regulated tissue specific gene expression confirming that these factors estab-lish distinct target gene programs. At the extremes, we found less than 10% of genes were among the top 500 in BRD4 enhancer signal (i.e. SE-regulated) and top 500 E2F promoter signal. We hypothesized that the presence of BETs and E2F in distinct regulatory axes divides active genes in MM into those that can be selectively influenced by BET inhibition or E2F perturbation, but not both. In line with this we have observed that dual E2F and BET inhibition is synergetic 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.

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Madrid, Spain, June 22 – 25, 2017
Aims: We used a custom target pulldown (TPD) approach on a large cohort of MM samples 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 genes, 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, NHRAS, TP53, FAMM6C, BRAF, DIS3, TPAF3, SPH40, IRS4) 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 NHRAS 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 1.25-1.81), amp(1q) (HR2.63, CI 1.92-3.59), del(17p), del(17p) with monosomy 13 and novel oncogenic copy number changes such as the high level amplification of MAF in 1 case.

Summary/Conclusions: We have developed and demonstrated utility of a reliable workflow for genome-wide copy number assessment that can be implemented using existing targeted short read sequencing data, greatly extending the utility of this technology beyond the identification of driver mutations in 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.

E1202
THE MULTIPLE MYELOMA GENOME PROJECT: DEVELOPMENT OF A MOLECULAR SEGMENTATION 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 prognostic 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 initiative that aims to define the landscape of genomic and cytogenetic alterations 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=1307), Whole Genome Sequencing (WGS; n=459), and expression data from RNAseq and CNA assays (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 standard methods and six clinical datasets. 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 dataset with various correlates. Based on our data, we have at least
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 novel small compounds that suppress growth of MM cell lines, and found that OSSL_325096 has strong anti-proliferative activity on MM cell lines (IC50 100-500µM). In this study, we evaluated anti-MM activity of OSSL_325096 through VCP inhibition, in an ATP-competitive manner.

Methods: OSSL_325096 were purchased from Princeton BioMolecular Research, Inc. (Princeton, NJ, USA). His-tagged human VCP (hVCP) cDNA was cloned and utilized to generate hVCP protein in vitro as previously described (Chou et al., PNAS, 2011, vol. 108(12): 4834-4839) to evaluate the VCP inhibition by OSSL_325096. For in vivo analysis, MM xenograft model mice were intraperitoneally administered with vehicle or 50mg/kg of OSSL_325096 twice a week.

Results: OSSL_325096 inhibited proliferation of MM cell lines, including one bortezomib-resistant cell line (Figure 1). OSSL_325096 induces apoptosis in these 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 in VCP’s D2 domain. Indeed, in the cell-free ATPase assay, OSSL_325096 showed dose-dependent inhibition of VCP’s ATPase activity (Figure 3). The IC50 of OSSL_325096 on ATPase activity was 7-10µM, while IC50 of cell survival in MM cells was 0.1-0.8µM, suggesting that OSSL_325096 may have other anti-myeloma function in addition to VCP inhibition. RNA-sequencing of MM cells treated with OSSL_325096 revealed that several pathways including mTRO1 signaling, TNFα signaling, and unfolded protein response, were activated by OSSL_325096. Finally, OSSL_325096 was administered to xenograft mice with MM cell tumors and inhibited the tumor growth in vivo (Figure 4).

Summary/Conclusions: The present data suggest that OSSL_325096 may be novel anti-myeloma drug candidate partially through its direct inhibition activity of VCP.

E1206
A NOVEL PREDICTIVE MODEL COMBINING LINCRNAS AND PROTEIN CODING GENES IN MULTIPLE MYELOMA
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Background: We have established the largest repository of molecular profiling data in MM associated with clinical outcomes. Integrated analyses are enabling generation of clinically meaningful disease segments associated with differing risk that will inform development of clinical tests. ThempP intends to build a global network by expanding collaboration with global MM centers to incorporate additional datasets through current and new collaborations.

*The first 6 authors share co-first authorship. The last 3 authors share co-senior authorship.
Background: RNA has diverse sets of regulatory functions and a recent analysis of a large RNA 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. lincRNA genes have been considered to provide regulatory functions, however, their precise role and function remains unclear. Aim: Here we have studied lincRNAs using uniformly treated patients to show their impact on survival outcome in MM. Methods: We performed RNA-seq on CD138⁺ MM cells from 360 newly-diagnosed patients and 18 normal plasma cells (NPM) and analyzed for lincRNA and protein coding genes. MM patient data included morphologic characteristics, cytogenetic and FISH as well as clinical survival outcomes were also analyzed and correlated with lincRNA data. Results: Using only the expressed lincRNAs, we developed a risk prediction signature from EFS at 4 years was 53% (95% CI, 45.1% to 63.1%) and 32.6% (95% CI, 25.1% to 42.2%), and OS at 4 years was 93.2% (95% CI, 88.9% to 97.6%) and 71.1% (95% CI, 62.9% to 80.3%) in our patients having a low or high risk score, We then compared lincRNA signature with known expression signatures and improved the risk prediction for known expression signatures dramatically. We validated our results on independent large cohort with newly diagnosed MM RNAseq data. When applied to patient cohort separated by other risk categorization including minimal residual disease status (MRD), cytogenetic risk status (del17p, t(4;14) and t(14;16)) and International Staging System (ISS), lincRNA signature was able to further identify patients with significant differential survival outcomes. Summary/Conclusions: In summary, we report that lincRNAs have an independent effect on survival outcome in MM and provides rationale for its use in risk stratification as well as to understand biological impact. Combined prediction with other risk features improve the prediction power and helps to create better classification in MM.

E1207

DYNAMIC IMMUNOHISTOCHEMICAL EVALUATION OF MARROW MICROENVIRONMENT MODIFICATIONS IN PATIENTS WITH SMOLDERING MYELOMA

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Background: In most cases, multiple myeloma (MM) is preceded by an asymptomatic status known as monoclonal gammopathy of unknown significance (MGUS) or smoldering multiple myeloma (SMM). The mechanisms of progression from SMM to MM are not well understood. Despite an increasing evidence of an immune system dysregulation in the setting of MM characterized by a tumor tolerating microenvironment, the immunosurveillance profile in the setting of SMM has never been investigated.

Aim: Our aim was to identify a progressive dysregulation of marrow microenvironment in sequential samples of SMM patients. Secondly, we hypothesized a difference in the microenvironment of the patients with progressed SMM versus those with stable SMM.

Methods: We performed extensive immunohistochemical analysis of bone marrow samples of 16 patients affected by SMM at time 0 (16 samples) and at +24 months (+/- 4 months, 16 samples). Half of these patients developed MM at 24 months (progressed SMM), the other half remained asymptomatic (stable SMM) at 24 months (+/−4 months, 16 samples). We aimed to characterise the immune landscape in the bone marrow of SMM and MM patients following ASCT, and to investigate the impact of residual disease on immune cell population. Results: At time 0, we found in SMM patients increased CD4+Foxp3− CD8 effectors, but high co-expression of LAG-3 suggests that these cells are functionally suppressed. Patients with larger amounts of residual disease have higher numbers of cytotoxic CD4 and CD8 cells, and the co-expression of the checkpoint protein LAG-3 may provide a rationale for blockade of this pathway.

Summary/Conclusions: The BM of MM patients following ASCT contains activated CD4 and CD8 effectors, but high co-expression of LAG-3 suggests that these cells may be functionally suppressed. Patients with larger amounts of residual disease have higher numbers of cytotoxic CD4 and CD8 cells, and the co-expression of the checkpoint protein LAG-3 may provide a rationale for blockade of this pathway.
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 interactions are 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 form MM patients (MM-BMSCs) and MGUS patients (MGUS-BMSCs) were isolated by the classical cell culture, and were homogeneously CD73+, CD90+, CD105+, CD34-, and CD45-.

Results: MM-BMSCs and mgUS-BMSCs had a fibroblast-like morphology in culture, and were homogeneously CD73+, CD90+, CD105+, CD34-, and CD45-. MM-BMSCs had a higher expression of α-smooth muscle actin (α-SMA) than mgUS-BMSCs. The nanoparticle size distribution of EVs derived from BMSCs was approximately 50 nm. We found high expression of miR-10a in the EVs derived from MM-BMSCs, while the expression of intracellular miR-10a was low in MM-BMSCs. We therefore hypothesized that low expression of cellular miR-10a might be important for survival of MM-BMSCs; As a result, miR-10a was packaged into EVs, and they were released to the extracellular space. To test the hypothesis, miR-10a mimic was transfected into BMSCs and the effect on EV secretion was investigated. Knockout of PBK was increased in cells expressing PBK and induced apoptosis of MM-BMSCs, while the cell proliferation and apoptosis of mgUS-BMSCs were not affected by the overexpression of miR-10a. We also found that inhibition of EV release with GW4869 promote the accumulation of intracellular miR-10a in MM-BMSCs, and EV-release inhibitor also can inhibited cell proliferation and induced apoptosis of MM-BMSCs.

Summary/Conclusions: Our findings indicate that expression of PBK and/or G9a/GLP represents new promising targets for the treatment of multiple myeloma. E. De Smedt1,*, J. Devin2, H. Liu3, A. Maes1, K. Maes1, K. De Veirman1, M. Menu1, K. Vanderkenken1, J. Moreaux2, E. De Bruyne1

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E1210 SINGLE-NUCLEOTIDE POLYMORPHISM IN THE PBK GENE IS CLOSELY ASSOCIATED WITH MYELOMA CELL PROLIFERATION I. Hanamura1, A. Ota2, S. Karna2, M. Wahiduzzaman2, S. Mizuno1, K. Uchino1, J. Kanasugi1, T. Horio1, S. Murakami1, S. Suzuki3, R. Ueda3, S. Tsuzuki2, H. Konishi2, Y. Hosokawa2, A. Takami1

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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.

Aims: The aim of this study is 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 human sequence of the whole PBK gene was determined using the dye terminator method. Knockout of PBK was performed using CRISPR-Cas 9 system. A single guide RNA sequence for PBK was selected by the CRISPR method. Knockout of PBK was confirmed by real-time RT-PCR and western blotting.

Results: In vitro, knockout of PBK significantly suppressed cell growth in vitro and in xenograft mice (Fig. 2). Moreover, exogenous expression of PBK increased cell proliferation in the PBK-deficient OPM2 cells, which carry PBK. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing PBK compared with those cells expressing PBK. KMS-11 cells carrying PBK knockout by CRISPR-mediated knockout suppressed cell growth in vitro and in xenograft mice (Fig. 2). Moreover, exogenous expression of PBK augmented cell proliferation in the PBK-deficient OPM2 cells, which carry PBK. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing PBK compared with those cells expressing PBK.

Summary/Conclusions: Our findings indicate that expression of PBK in myeloma cell proliferation, while PBK in MM was likely related to tumor suppression. Increased phosphorylation of Thr 9 on PBK might contribute to proliferation in cells with PBK. These results provide a novel insight into the mechanisms underlying myeloma cell growth and PBK is a potential stratification and therapeutic target for plasma cell dyscrasias.

E1211 THE HISTONE METHYLTRANSFERASES G9A/GLP REPRESENT NEW PROMISING TARGETS FOR THE TREATMENT OF MULTIPLE MYELOMA E. De Smedt1,*, J. Devlin2, H. Liu3, A. Maes1, K. Maes1, K. De Veirman1, M. Menu1, K. Vanderkenken1, J. Moreaux2, E. De Bruyne1

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Background: Multiple myeloma (MM) is a clonal plasma cell malignancy which mainly resides in the bone marrow. In cancer cells, the epigenetic landscape is known to be highly disturbed. In line, numerous epigenetic aberrations have been described in MM, resulting in deregulated gene expression, disease progression and drug resistance. Targeting deregulated epigenetic modifiers therefore represents an interesting therapeutic approach. G9a (EHMT2) and GLP (EHMT1) are 2 histone methyltransferases which catalyze mono- and dimethylation of histone 3 lysine 9 (H3K9). Importantly, G9a is overexpressed in several cancers, correlating with a poor prognosis.

Aims: Currently, data about the expression and role of G9a/GLP in MM is lacking. The aim of this study is therefore to investigate the functional role of G9a/GLP in MM pathogenesis.

Methods: The prognostic value of G9a/GLP in terms of overall survival was determined in the UAMS-TT2 cohort of newly diagnosed MM patients (n=345, 2012-2014) and survival was analyzed. In addition, we used a panel of 10 human cell lines, 3 murine cell lines and 5 primary patient samples to evaluate the effect of the small molecule inhibitors UNC0638 and BIX01294 on MM cell viability, cell cycle progression and apoptosis. We also assessed the in vitro anti-MM activity of BIX01294 in combination with bortezomib or ABT-199.

Results: In vitro anti-MM activity of therapeutic BIX01294 treatment was tested using the murine 5G1 cell line. Difference in overall survival between groups was assessed with a log-rank test and survival curves plotted using the Kaplan-Meier method.
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 immunosuppression, notably the PD-1/PD-L1 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 functional capacity. Dimensionalisation 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 TGFβ. The expression of G9a/GLP are associated with a worse disease outcome in newly diagnosed MM patients. Moreover, gene set enrichment analysis of patients with high G9a/GLP expression levels displayed a significant enrichment of genes involved in pathways associated with MM disease progression, including the RAS pathway, NF-kB canonical pathway, IRF4 multiple myeloma program and mRNA splicing. Sequencing data shows somatic mutations in specific G9a/GLP inhibitors BIX01294 and the UCN0138 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 altered the tumor microenvironment as evidenced by 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 5TM1 inoculated mice with BIX01294 resulted in a clear antitumor effect, 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. Furthermore, specific targeting of G9a/GLP induces MM cell apoptosis, enhances MM sensitivity to ABT-199 and bortezomib and significantly delays tumor progression in the murine 5TM1 model. Thus, G9a/GLP targeting represents a promising strategy to improve treatment of MM.

E1213
P53-RESTORING SMALL MOLECULE CP-31398 INDUCES APOPTOSIS VIA INDUCTION OF REACTIVE OXIDATIVE SPECIES IN HUMAN MULTIPLE MYELOMA
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Background: Reactive oxygen species (ROS) are normal byproducts of a wide variety of cellular processes. ROS have dual functional roles in cancer cell pathophysiology. At low to moderate levels, ROS act as 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 tumor-associated p53 mutants to wild type p53 function in multiple human cancer cell lines; this leads to cell cycle arrest and/or apoptosis. Although the growth of rhodomyeloblastoma 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, KMM15) and two primary myeloma samples were used. CP (200 nM) was added 48 h subsequent to treatment; consequently, the inhibitory effect of CP on MM cell line growth was assessed using a WST-1 assay. In order to elucidate the cytotoxic mechanism of CP, immunoblotting and flow cytometry analyses 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 2.51–11.2 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 may satisfy an unmet clinical need, as such patients currently have a poor prognosis.

E1214
TUMOR MICROENVIRONMENT TRANSFORMATION FROMmUS TO MYELOMA IS ASSOCIATED WITH PRO-TUMORAL ACTIVATION OF MESOTHERMAL STROMAL CELLS (MSCS)
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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 demonstrated that MM-MSC co-culture is able to convert normal immature myeloid cells in MM-derived MSC 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 tumor microenvironment transformation. Methods: Human peripheral blood mononuclear cells (PBMC) isolated from SMM and MM were co-cultured with healthy controls (HC)-mUS-, MM- or MM-MSC. After 6 days, neutrophils (N) were isolated using anti-CD66b magnetic microbeads and were tested in vitro for their ability to induce angio-genesis and suppress T cell proliferation.
Results: Only N educated by SMM- and MM-MSC (both from patients at diagnosis, relapsed and refractory) significantly up-regulated Arg1, NOS2 and TNFα and exhibited suppressive effect with a reduction of T cell proliferation (p<0.001). By co-culturing educated-N with Human Brain Microvascular Endothelial Cells (HBMEC), we observed increased both tube length and number of branch points only in conditions where HBMEC were incubated with MM-MSC (MM-MSC+HBMEC) vs. SMM-MSC+HBMEC (n=3, p<0.05). Adding Bortezomib, Lenalidomide or Pomalidomide during co-culture of PBMC with MM-MSC, isolated N showed a significant reduction of pro-angiogenic activity but did not lose immunosuppressive ability. To examine if PC play a role in MSC “activation”, before performing co-cultures with PBMC, we pre-treated HS-S or HC-SCM with MM cell lines. PC pre-treatment drives a healthy MSC to activate N in immunosuppressive and pro-angiogenic cells. Implantaion of mixtures of fluorescently labeled MM cells and healthy- or MM-MSC into zebrafish, animals cojected with PC and MM-MSC showed enhanced tumor colonization and growth compared with those injected with PC and healthy MSC.

Summary/Conclusions: Character of tumor microenvironment transformation frommmUS to MM is associated with progressive activation of MSC that have a pro-tumoral activity. Indeed SMM- and MM-MSC polarize N in immunosuppressive and pro-angiogenic N (NZ) in vitro. In addition, MM-MSC facilitate MM growth in vivo confirming their central role in tumor progression.

E1215

LONG TERM CR MULTIPLE MYELOMA PATIENTS STUDIED WITH NEXT GENERATION FLOW SHOW PREDOMINANTLY CURED VSmgUS-LIKE MINIMAL RESIDUAL DISEASE PATTERNS: A STUDY OF THE GTMM-TUSCAN GROUP FOR MULTIPLE MYELOMA

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Background: Angiogenesis is a hallmark of tumors, and it is a peculiar characteristic in bone marrow (BM) of multiple myeloma (MM) patients. MM is still a incurable disease that strongly depends on interactions with BM microenvironment (TME). Endothelium of MM patients displays malignant behavior as compared to a healthy counterpart (1). MM displays a dysregulation of the Notch pathway due to Jaged ligands and Notch receptors overexpression. This condition brings about the generation of homotypic and heterotypic interaction loops that sustain MM growth. Moreover, Notch signaling is able to suppress BM resident cells, including osteoclast and BM stromal cells (BMSCs), although its role in the crosstalk of MM and endothelium is still to be clarified.

Aims: The aim of this study is to investigate Notch role in MM crosstalk with endothelium exploiting 2D assays and 3D organoid systems to mimic tumor microenvironment (TME).

Methods: The Notch ligands, Jaged1 and 2, were silenced in the MM cell line RPMI8226 (RPMI8226-TruJADG1/2 ) using an inducible lentiviral vector carrying two short hairpin RNAs targeting Jaged1 and 2. To mimic the endothelial compartment, both early and late endothelial cells (HAECs) were used and for the stromal compartment, the GFP+HSS cell line. Matrigel and collagen healing assays were set up to investigate Notch role in modulating the angiogenic potential of MM cells co-cultured with HAECs and HAECS motility in response to MM-derived soluble factors. To develop a TME-like system, a decellularized extracellular matrix (dECM) was used as a physiologic scaffold for organoid generation. dECM was produced by treating murine fibroblast NIH3T3 with ascorbic acid and was loaded with cells for organoids generation. We evaluated apoptosis of MM cells in single culture and co-culture with BMSCs or HAECS by flow cytometry.

Results: Matrigel assay of HAECs co-cultured with MM cells showed that direct contact increased angiogenic potential of HAECs to form a grid of tubes; this effect is significantly reduced when HAECs are co-cultured with RPMI8226-TruJADG1/2 cells, indicating a key role of Notch signaling in endothelial stimulation. Wound healing assay demonstrated that Notch signaling affects MM cells mobility, since it is reduced when Jaged ligands are ablated. Concerning the 3D-organoid generation, our results indicate that the handcrafted dECM was a suitable scaffold. Moreover, apoptosis assays indicated that MM cells displayed an increased survival when cultured in the presence of BMSCs, that consistently with their recognized protective role; no significant difference in MM cell apoptosis was observed in the presence of endothelial cells. On the contrary, we have observed that endothelial cells were protected by MM cells suggesting that MM cells improve angiogenesis by preventing endothelial cells apoptosis.

Summary/Conclusions: These results indicate a novel role for Notch pathway in MM-EC crosstalk suggesting that the Notch pathway activation in MM cells can increase their proangiogenic potential. 3D-organoid mimics BM microenvironment and may be used as a novel tool to recapitulate the interactions of BM and tumor cells beyond the animal models.

References

E1217

MIR-101-3P REGULATES BONE MARROW STROMA-INDUCED DRUG RESISTANCE IN MULTIPLE MYELOMA CELLS BY TARGETING SURVIVIN AND MODULATING CELL-CELL ADHESION

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Background: In multiple myeloma (MM), bone marrow stromal cells (BMSCs) protect MM cells against cell death by direct or indirect interaction. This phenomenon is poorly understood. We partly explain de novo or acquired drug resistance in MM. Findings of relevant studies indicate activation of some oncogenic or survival pathways including PI3K/mTOR, Ras/MAPK, NFκB and Wnt. However, the potential regulatory mechanisms and druggable targets have not been clearly elucidated.

Summary/Conclusions: In conclusion NGF showed that MM patients with a CR can have long lasting remissions meaning disease control. Patients in sustained CR after 2 years can have high percentage of MRD negativity. Larger studies are warranted to identify patients who need treatment consolidation or continuous treatment based on MRD status vs others who could stay treatment free with social and economical benefits.
**Aims:** To understand the role of stromal induced drug resistance and to identify new therapeutic target 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 assays (BMMSCs) were then seeded on BMMSCs in presence or absence of BTZ. GFP fluorescence-based adhesion, cytotoxicity and annexin-V/PI apoptosis were applied.

**Results:** qPCR arrays showed that BMMSCs up- or down-regulated several miRNAs and mRNAs in MM cells. Survivin (BIRC5) was confirmed to be conserved in JNJ3 cell lines and mRNA and protein expression in monolayers. In contrast, miR-101-3p was confirmed to be significantly downregulated by stroma in MM cells. Moreover, suppression of miR-101-3p or upregulation of survivin was reversed partially when BMMSCs were pre-treated with BFA but highly significantly when they were separated from MM cells with a TW insert. The same trend was observed in in vivo results FACS analysis indicating that direct cell-cell adhesion was more effective in BMMSC-induced modulations in MM cells. Next we identified that survivin was a direct target of miR-101-3p, overexpression of miR-101-3p suppressed survivin mRNA/protein. As indicator of involvement in stroma-mediated drug resistance, survivin and miRNA-101-3p 3p inhibition of the bone marrow niche was thus effective in BMMSC 3p cultured. Furthermore, miR-101-3p overexpression or silencing of survivin increased BTZ-induced apoptosis in MM cells in the absence or presence of BMMSCs significantly overcoming stroma-mediated drug resistance. To test whether miRNA-101-3p could also regulate adhesion of MM cells to BMMSCs, we further 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-BMSC adhesion.

**Summary/Conclusions:** Our results identify a mechanism whereby BMMSCs induce drug resistance in MM cells by up-regulating survivin and down-regulating 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 axis in MM cell growth, progression and drug resistance.

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**E1218**

**ARQ-197, A SMALL-MOLECULE INHIBITOR OF c-MET, REDUCES TUMOUR BURDEN AND PREVENTS TUMOUR-ASSOCIATED BONE DISEASE IN A MURINE MODEL OF MM**

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**Background:** The receptor tyrosine kinase c-Met, its ligand HGF, and their signaling have been implicated in the pathogenesis of myeloma. In myeloma patients with elevated levels of HGF its prognostic is known to be poor. Therefore, targeting these molecules or their pathway in such patients may be of great benefit. We hypothesised that ARQ-197 (Tivantinib), a small molecule c-Met inhibitor, would reduce myeloma cell growth and prevent myeloma-associated bone disease in a murine model.

**Aims:** 1. Assess effects of ARQ-197 on myeloma cell proliferation. 2. Assess effects of ARQ-197 treatment on tumour burden in murine models of myeloma and 3. Assess effects of ARQ-197 treatment on myeloma bone disease in murine models of myeloma.

**Methods:** In vitro we assessed the effects of ARQ-197 (0.1563 μM - 5 μM) on myeloma cell proliferation, cytotoxicity and c-Met protein expression in the JNJ3 human cell line. In vivo we intravenously injected NOD/SCID-γ mice with 10^6 JNJ3 cells and 1 week later treated mice with either ARQ-197 (200mg/kg/day, 5 times per week by oral gavage) or vehicle for 2 weeks. Response to drug resistance in MM cells by upregulating survivin and GSK3 in a significant inhibition of cell proliferation (p<0.001) and an induction of cell death (p<0.001), probably caused by significantly [SL1] reduced levels of phospho-c-Met. In vivo ARQ-197 treatment of JNJ3 tumour-bearing mice resulted in a significant reduction in tumour burden (p<0.001), where tumour infiltration by ARQ-197 was reduced by approximately 43% (864±9% vehicle vs 55±20% ARQ-197 treatment). ARQ-197 treatment also significantly prevented the formation of myeloma-induced bone lesions (P<0.001) and the loss of trabecular bone (p<0.01) compared to vehicle treated JNJ3-tumour bearing mice. Dynamic histomorphometry showed ARQ-197 treatment prevented bone loss in the mineralizing bone surface (p<0.001), the mineral apposition rate (p<0.01), the bone formation rate (p<0.01), and prevented complete loss of osteoblasts on the cortico-endosteal bone surface compared to the vehicle group.

**Summary/Conclusions:** In summary, these results suggest that ARQ-197 could be a promising therapeutic in myeloma patients who express high levels of HGF, leading to both a reduction in tumour burden and an inhibition of myeloma-induced bone disease.

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**E1219**

**Abstract withdrawn.**

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**E1220**

**THE GENETIC LANDSCAPE OF THE MURINE 5T MODELS FOR MULTIPLE MYELOMA**

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**Aims:** Multiple myeloma (MM) is a plasma cell malignancy which remains incurable in most cases. This is mainly attributed to the large genetic and epigenetic heterogeneity of MM patients. However, to date, the genetic landscape of murine MM models have not been analyzed. Our aim of this study is to analyze the genetic landscape of the 5T murine models for MM.

**Methods:** In this study, we used the 5T2, 5T33vv and 5TGM1 murine models for MM. As control samples, we used C57BL/KeLwRj and C57Bl/6j germine DNA. We analyzed the copy-number alterations and the mutualional landscape using whole shell genome sequencing and whole exon sequencing.

**Summary/Conclusions:** Among the tested models, the 5T2 model displayed the most copy number alterations. Over the entire genome, 11% and 17% showed copy number alterations for the 5T33vv and 5TGM1 of which 6% is shared reflecting their clonal relationship. Overall, the copy-number alterations affects genes involved in RAS/MAPK, PI3K/AKT1 and JAK/STAT signaling. DNA damage response, cell cycle and epigenetic regulation. Exome sequencing revealed the presence of 417, 407 and 314 non-synonymous mutations and 8, 14 and 24 indels in the 5T33vv, 5TGM1 and 5T2 models, respectively. Moreover, a statistically significant overlap of mutated genes between the 5T33vv and 5T2 models was observed (p<1E-8). Similar to MM patients, we identified damaging mutations in Trp53, Rb1, Pik3ca, Fat3, Kdm6a and Nf1.

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**E1221**

**CHARACTERIZING THE CONTRIBUTION OF BONE MARROW Stromal-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 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 stem/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, mgUS, and MM patients and used our “humanized” bone marrow-like model to characterize the molecular impact of MM cells on BMMSCs. Finally, we have validated targets by generating HS-5 knock-out lines using CRISPR/Cas9 targeting.

**Results:** Analyzing the BMMSCs of healthy individuals, mgUS, and MM patients and used our “humanized” bone marrow-like model, allowed us to confirm established disease biomarkers (e.g. IL-
6, HGF, IGF and GDF15) and identify novel mediators of MM disease progression and bone disease. To further elucidate the role of IL-6 in BMSC-induced growth of MM plasma cells and drug resistance, we have established HSS stromal cell lines that upon CRISPR/Cas9 targeting have reduced or no expression of IL-6. RNA sequencing analysis of these cells revealed IL-6 to be a master regulator of cytokine production (e.g. IL-1β, CXCL8, CSF-2 and CSF-3). Disruption of the IL-6 gene did not result in a reduced growth rate of the IL-6 deficient stromal cells as compared to wildtype. Using the compartment specific bioluminescent imaging co-culture system, where luciferase gene-marked MM cells are co-cultured with non-marked stromal cells, we have documented a contribution of the stromal cells to both growth and drug resistance to known chemotherapeutics (e.g. bortezomib, doxorubicin) of MM cells. Using this same co-culture system we compared wildtype and IL-6 deficient stroma. Although disruption of IL-6 in the stromal cells resulted in a reduced proliferation of MM cells and stromal cell mediated drug resistance, it did not entirely reverse these stroma-mediated effects.

**Summary/Conclusions:** Taken together these data suggest that although IL-6 is one of the most deregulated genes in MM-derived BMSCs, it certainly is not the sole contributor to BMSC-induced MM cell growth and drug resistance.

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**E1222**

**THE PAN-PIM KINASE INHIBITOR, PIM447, POTENTLY SYNTHETIZES WITH POMALIDOMIDE PLUS DEXAMETHASONE IN PRECLINICAL IN VITRO AND IN VIVO MODELS OF MULTIPLE MYELOMA**

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**Background:** PIM kinases are a family of serine/threonine kinases recently proposed as therapeutic targets in multiple myeloma. Recent work from our group has shown the dual antitymoma and bone-protective effects of the pan-PIM kinase inhibitor, PIM447, and its in vitro synergism with current standards of care. Since myeloma remains an incurable disease, the preclinical evaluation of new drug combinations is of utmost importance, in order to support the development of future clinical trials. In this scenario, effective all-oral combinations are particularly attractive.

**Aims:** The aim of the present work has been the evaluation of the efficacy and mechanism of action of the all-oral triple combination PIM447 + pomalidomide + dexamethasone in preclinical in vitro and in vivo models of multiple myeloma.

**Methods:** In vitro cytotoxicity of PIM447, pomalidomide and dexamethasone alone or in double and triple combinations was evaluated on myeloma cell lines. The combination index (CI) was calculated with CalcuCyxus software based on results from MTT assay. Effects on apoptosis and cell cycle were evaluated by flow cytometry. Glucose uptake was analyzed by incubation with 2-NBDG. The mechanism of action was explored by analysis of different protein levels by western blot. Finally, a plasmacytoma model in CB17-SCID mice was employed for in vivo studies.

**Results:** Triple combination PIM447 + pomalidomide + dexamethasone showed a strong synergism (CI<0.3) in M15HS and RPMI-8226 cell lines. The cytotoxic effect was increased in the presence of PIM447 and pomalidomide, promoting a downstream reduction of the glucose metabolism-associated enzyme hexokinase II and also reduced glucose uptake by cells. Finally, the efficacy of this combination was confirmed in a plasmacytoma model in CB17-SCID mice, where it clearly reduced tumor growth as compared to single and double treatments.

**Summary/Conclusions:** Our preclinical data suggest that myeloma patients could benefit from treatment with the triple combination PIM447 + pomalidomide + dexamethasone and would support future clinical trials with this combination.

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**E1224**

**TRIM33 IS A POTENTIAL TUMOR SUPPRESSOR IN MULTIPLE MYELOMA**

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**Background:** Multiple myeloma (MM) continues to be an incurable plasma cell neoplasm, regardless of recent therapeutic advances. The success of proteasome inhibitors in MM validates the ubiquitin proteasome system (UPS) as a therapeutic target. Using a UPS-specific microarray (PIQOR) we identified aberrant expression of an E3 ligase TRIM33 (tri-partite motif containing protein 33) in 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 and/or immunofluorescence montage and in situ analysis of 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 shRNAs elicited with Lentivirus. The knockdown efficiency was verified by qPCR.

**Results:** Knockdown of TRIM33 expression was achieved in all cell lines, with varying levels of knockdown: J3N3 (p<0.0001) 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 in patients with a t(4;14) compared to other MM subtypes, particularly (6;14) (p=0.004) and hyperdiploid cluster (p=0.05). Low TRIM33 expression has also been associated with poor overall survival (GSE2658; p=0.0034). Forty-seven patients were treated with TRIM33 expression in the non (t(4;14) and (t(4;14) MM were identified; these genes were analysed using QUADRATIC tools connectivity mapping to identify FDA approved drugs predicted to enhance the TRIM33 gene signature. One of the top enhancers identified was the tyrosine kinase inhibitor (TTK) Imatinib. The OPM-2 cell line showed greatly increased sensitivity to Imatinib compared with other MM subtypes (p<0.05) and U266 (p<0.005). Analysis of a publically available dataset to look at TRIM33 expression and correlation with survival in subsets of newly diagnosed MM; GSE19784 (N=320) and GSE2658 (N=551). qPCR was used to validate the changes in expression of the TRIM33 gene signature.

**Results:** Compared to normal bone marrow, lower expression of TRIM33 was observed at both gene and protein level (p=0.03) in the (t(4;14) cell lines, KMS-18 and OPM-2. Conversely, expression was found to be high in the non (t(4;14) cell lines, JNJ3 (p=0.001) and U266 (p=0.015). Knockdown of TRIM33 expression 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 in patients with a t(4;14) compared to other MM subtypes, particularly (6;14) (p=0.004) and hyperdiploid cluster (p=0.05). Low TRIM33 expression has also been associated with poor overall survival (GSE2658; p=0.0034). Forty-seven patients were treated with TRIM33 expression in the non (t(4;14) and (t(4;14) MM were identified; these genes were analysed using QUADRATIC tools connectivity mapping to identify FDA approved drugs predicted to enhance the TRIM33 gene signature. One of the top enhancers identified was the tyrosine kinase inhibitor (TTK) Imatinib. The OPM-2 cell line showed greatly increased sensitivity to Imatinib compared with other MM subtypes (p<0.05) and U266 (p<0.005). Analysis of a publically available dataset to look at TRIM33 expression and correlation with survival in subsets of newly diagnosed MM; GSE19784 (N=320) and GSE2658 (N=551). qPCR was used to validate the changes in expression of the TRIM33 gene signature.

**Summary/Conclusions:** We have shown that TRIM33 exhibits lower expression in (t(4;14) cell lines, compared to non (t(4;14) cell lines and that knockdown of TRIM33 increased the viability of non (t(4;14) cell lines. This suggests that TRIM33 may act as a tumor suppressor in MM and that expression is dysregulated in a subset of MM. Connectivity mapping identified Imatinib as an
enhancer of the TRIM33 signature that potently decreased the viability of the OPF2 cell line. This study suggests that enhancing the TRIM33 gene signature could potentiate the tumor suppressive effect of TRIM33 and identify novel therapies for this subset of MM.

E1225
LONG NON-CODING RNAs EXPRESSION HETEROGENEITY AND FUNCTIONAL INVOLVEMENT IN MULTIPLE MYELOMA
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Background: Increasing amount of evidence indicates that deregulation of long non-coding RNAs (lncRNAs) is a common feature of cancer and therefore, its investigation may uncover new molecular oncogenic mechanisms. In multiple myeloma (MM), altered expression of small number of lncRNAs has been associated with decreased disease-free and overall survival, suggesting that these elements may play a more important role in MM than previously anticipated. Nevertheless, an extensive high-throughput analysis that characterizes the deregulation of lncRNAs in MM has not yet been performed.

Aims: We aim to characterize the IncRNA transcriptome of MM and its heterogeneity, and determine whether altered IncRNAs have a functional involvement in this disease.

Methods: Paired-end strand-specific RNA sequencing (ssRNA-seq) was performed in 38 purified plasma cell (PC) samples from MM patients, as well as in 5 tonsil PCs (TPCs) and in 3 bone marrow PCs (BMPCs) of healthy donors as controls. We also performed ssRNA-seq of populations from B cell differentiation (Naive, Germinal Center, Memory and PC). To study the heterogeneity of IncRNAs expression we performed sample level enrichment analysis (SLEA), in which each individual IncRNA was compared to BMPCs. To determine the epigenetic regulation of IncRNAs we used whole-genome bisulfite sequencing and CHIP-seq. shRNA-mediated knockdown using 2 different shRNAs and MET-INV conversion V (cell death) assays were utilized to study the functional effect of IncRNA overexpression.

Results: We identified 40.552 novel IncRNAs in MM samples that were present in at least 3 of the 38 patients. Principal component analysis demonstrated that TPCs and BMPCs cluster separately, suggesting that, in spite of being of the same cell type, their transcriptomes are very different. We observed that the expression of IncRNAs was more heterogeneous than that of coding genes. More importantly, SLEA showed 11.067 IncRNAs that were overexpressed and 5.601 underexpressed in >40% of patients. Thus, the number of deregulated genes analyzed by SLEA was much larger than the 70 IncRNAs that appeared as deregulated when all MM were compared to BMPCs, 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 Intergenic Long non-coding RNA), a IncRNA that it is overexpressed in ~ 40% of MM patients and not observed in the compartment of normal plasma cells (PCs) viability and movement. Finally, we analysed the in vivo effects of Eph3A/mAb in a MM mouse xenograft model.

Methods: Eph3A 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 Eph3A targeting by lentiviral RNA silencing (shRNA) and anti-Eph3A monoclonal antibody (Eph3AmAb) in vitro and in vivo were studied by adhesion assay on fibronectin on bone marrow stromal cells (BMSCs), invasion assays and proliferation MTS assay, respectively. Gene expression profiling (GEP) was performed in shEph3A versus shControl cells. Furthermore, the effects of Eph3AmAb 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: Eph3A was found overexpressed in primary MM BMPCs and MM cell lines when compared with HCs (figure 1A-B). The Eph3A loss of function by siRNA and by Eph3AmAb 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 Eph3A modulated some molecules that regulate adhesion, migration and invasion processes. Importantly, the treatment with Eph3AmAb in vivo significantly reduced tumor size and inhibited angiogenesis, as revealed by decrease of CD31+ vessels at immunohistochemistry (data not shown).

Background: The tyrosine kinase Eph receptor A3 (EphA3) has recently emerged as a potential therapeutic target, since it is 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 (Val 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-EphA3 monoclonal antibody (EphA3mAb) in vitro and in vivo were studied by adhesion assay on fibronectin 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.

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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.
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 57 years (30-81), we performed FISH with locus-specific and centromere DNA probes (XL 1p32/Iq21, XL IGH probe, 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 classes was initiated for 131 pts, 3 pts with smoldering MM remained under observation. Response was evaluated according to the IMWG criteria for 127 pts, because 4 pts died in induction. 48 pts were underwent ASCT. The median follow-up of group was 19.3 months (3.2 – 77.4). Progression was diagnosed in 69 pts, in 12 of them FISH-analysis was performed also in disease progression.

Results: Chromosomal aberrations were revealed in 133 of 134 (99%) pts. TlgH/Iq23 was detected in 42.5% (57/134), hypodiploidy in 57.5% (77/134), hypodiploidy in 2.4% (3/134) pts. In 11.2% (15/134) a concurrent tlgH/Iq23 and a trisomy were found. The IGH translocations t(11;14), t(4;14), t(14;16), t(14:20), t(6;14) were observed at a frequency of 16.4%, 12.7%, 3.7%, 2.2%, 0.7% respectively, chromosomal partner is not found in 6.7%. Del(13q) was detected in 40.3% (54/134), del(17p) in 12.7% (17/134), ttcMYC/8q24 in 17.2% (23/134). Amp1q21 was detected in 39.6% (53/134). We identified 3 copies of 1q21 in 60.4% (80/134), del(13q) was detected in 3% (4/134), 4-7 copies 1q21 (4-7) in 21 (39.6%) pts. Cases with amp1q21 had a high incidence of del(13q) (OR=2.71 (1.32-5.55); p=0.006) and 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: OR – 11.8% versus 14.5%, VGPR – 39.2% versus 27.6%; PR – 37.2% versus 27.6%; therapy resistant 11.8% versus 30.3% (p=0.07). Pts with amp1q21 had significantly worse 5-year overall survival (OS) (43.5% vs 79.4%; p=0.07). According to copy number of 1q21 the 5-year OS pts carrying 3 or >3 copies of 1q21 were 67.3% and 20.9% (p=0.0016) (Figure 1). On multivariate analysis >3 copies of amp1q21 (HR=4.29, p=0.0094), TTCMYC/8q24 (HR=6.51, p=0.0082), del(13p) (HR=3.46, p=0.007) were found to be an independent adverse predictors of shorter OS.

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.

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 using autoanalyzer and turbidimetric SFLC were performed using the Freelite assay on the SPARus 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 gammapathy 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), TCD8 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=0.09). No clearcut correlations were found between reductions in uninvolved Ig or 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 high frequency of reduction was in B lymphocytes and NKT cells, in keeping with reduced levels of circulating B cells, followed by T cells, particularly TCD8 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 enzyme activity and has well-defined roles in T cell activation and several tumor developments, including malignant lymphoma. However, little is known about the role of CD26 in regulating bone remodeling.

Aims: In this study, we examine the CD26 expression in human normal OCs and OCs of MM patients. We explore the function of CD26 in osteoclastogenesis of OCs 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 GSK2606414 for OC formation for TRAP staining and functional assay. To assess the mechanisms of action of CD26Ab on OC, 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. msFH-01, and sRANKL induced human OC differentiation, in association with CD26 expression on monocyte-macrophage lineage cells. CD26 expression was accompanied by increased phosphorylation of MKK3/6 and p38MAPK, which is crucial for human OC differentiation with its downstream activation of microphthalmia-associated transcription factor (mitf). Mitf plays an important role in OC functions. CD26 expression was evidenced as the number of multineucleated OCs (>3 nuclei) by TRAP/CD26 staining and down-regulated the secretion of TRAP-5b and type 1 collagen. It decreased the size of OCs and the number of nuclei per OC, with significantly defective bone resorption activity. It was revealed that in the presence of CD26Ab, which blocked OC precursor cells (OCPCs) CD26 expression and phosphorylation pathway was specifically, rapidly inactivated and subsequently, its downstream mitf 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-mitf was not phosphorylated at all in immature 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 NFXB was rapidly induced in response to RANKL both in OC precursor cells and OCs. However, the absence of phosphorylation at CD26 in 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 CD26-stained OCs were performed. We examined the expression of CD26 in MM cells. Although CD26 expression was only slightly detected in any of MM cell lines in mono-culture, CD26 expression level was upregulated in all MM cell lines, co-cultured with OCs by flow cytometry and immunohistochemistry. CD26 protein level in these cell lines was also increased by immunoblotting or ELISA. To further explore the CD26 expression in the BM microenvironment, we performed the upregulation of CD26Ab on CD26Ab stained MM cells. We also found that CD26Ab impairs the development of human functional OCs. Targeting CD26 positive OCs and certain endothelial vascular cells in several cases. Anti-myeloma efficacy of CD26Ab on MM cells, co-cultured with OCs was also evidenced.

Summary/Conclusions: Our data imply that the blockade of CD26 signaling with CD26Ab impairs the development of human functional OCs. Targeting CD26 in both OCs and MM cells with CD26Ab may be a promising novel therapeutic strategy in MM-associated bone disease and MM progression.

E1231

THE ANTI-MYELOMA ACTIVITY OF PERK KINASE INHIBITOR IN TARGETING MORE THAN 50 UPR-RELATED GENES INVOLVED IN THE PROLIFERATION OF MM CELLS

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Background: Due to the immunoglobulin production, multiple myeloma (MM) plasma cells are dependent on the unfolded protein response process (UPR), which controls protein production and ensures its proper translation and folding. A study by Michallet et al (2011) showed that knockdown of one of the three well-known arms of the UPR, PERK (protein kinase R (PKR)-like ER kinase) in MM cells resulted in autophagic cell death. This outcome indicated the importance of PERK activation for the maintenance of plasma cell to myeloma cell but also its ability to impede the apoptotic effect. In this study we used a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding site domain while displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.

Aims: In this study we aimed to use a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding domain, while displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.

Methods: We initially screened 25 CD138+ MM patients and 6 human myeloma cell lines (HMCLs) for PERK mRNA expression. Our results showed that PERK mRNA is highly expressed in almost all patients (5-10 fold higher than the mean PERK expression of HMCLs).

Results: To test the effect of GSK2606414 on the proliferation of MM cells, 4 HMCLs were treated with different doses of GSK2606414 at two time points (24 and 48 hours). Treatment of cells with 3-30μM GSK2606414 resulted in a dose-dependent inhibition of cell proliferation in all HMCLs ranging for 20-95% reduction of proliferative activity, thus, indicating the dependency of these cells on PERK signaling. Treatment of KMS11 and KMS12 cells with 3-30μM GSK2606414 for 24 and 48 hours resulted 25% and 15% increase in apoptotic cells by Annexin-V staining, respectively compared to untreated cells. In the absence of PERK inhibition, the percentage of apoptotic cells was increased in 40% and 30% reduced cell proliferation in H929 and L363 cells respectively compared to control. The effect of GSK2606414 in combination with bortezomib in the proliferation of H929 and L363 cells was examined. As seen in the apoptosis assay, pre-treatment of these cells with 10μM bortezomib for 24 hours increased the percentage of apoptotic cells by 40-50%. In addition, GSK2606414 and 10μM bortezomib combined treatment resulted in 70% and 40% reduction of cell proliferation in H929 and L363 cells respectively compared to control.

A study by Michallet et al (2011) showed that knockdown of one of the three well-known arms of the UPR, PERK (protein kinase R (PKR)-like ER kinase) in MM cells resulted in autophagic cell death. This outcome indicated the importance of PERK activation for the maintenance of plasma cell to myeloma cell but also its ability to impede the apoptotic effect. In this study we used a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding domain while displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.

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**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 ONCO-METABOLIC CHECKPOINT

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**Background:** The bone marrow (BM) environment plays a crucial role in the incurable plasma cell (PC) malignancy multiple myeloma (MM). Our previous work showed that arginine deprivation (arginine/glutamine <1000:1000) was established long-term and PC fitness, regulating Blimp-1, immunglobulin (Ig) production and ATP availability (Penge, 2013) and that MM is addicted to the autophagy receptor SQSTM1/p62 (Miller, 2015). Among putative pro-tumoral patients are immunosuppressive BM-derived high-density neutrophils (HDNs), but the role of HDNs 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, 30 healthy, 30 healthy controls), (2) metabolomic profiling by UHPLC/GC-MS of ad hoc collected BM and peripheral plasma (16 MM, 17 smoldering MM, 30mgU, 29 controls), and (3) functional and expression 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 sequenc- (RNA-seq) available from the open-access, public clinical and molecular database, the CoMMpass Researcher Gateway (RG, https://research.themm.org, v I8A, n=649).

**Results:** In vitro, selective and progressive arginine deprivation (range 1000-0 μM) in four MM cell lines (MS 1S, U266, OPM2 and RPMI828) activated the GCDN/CHOP axis, resulting in increased p62 and Blimp-1 expression, increased ATP availability and immunglobulin production. Conversely, stable lentiviral p62 silencing significantly reduced Blimp-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 Blimp-1 expression and eGFR. The percentage of patients with abnormal eGFR decreased as p62 transcriptionally predicted shorter progression-free survival at 24 months (4.56 months versus 11.6 months, p<0.0001).

**Summary/Conclusions:** Taken together, our findings disclose a novel envi-ronmental circuit co-opted by MM pathway, wherein immunosuppressive HDNs sustain PC fitness through arginine depletion to increase p62 and Blimp-1 via the GCN2/CHOP pathway.

**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 trans-plantation and novel agents has improved early mortality rates (EM, henceforth defined as death within one 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 regres-sion 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 κ2 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 (t<04ml/min/1.73m²) was higher in the EM group compared with the rest of the patients (69% vs 17%, p<0.001). In accordance with the International Staging System (ISS), advanced MM stage (i.e. ISS3) was observed more often in the EM group compared to the rest (65% vs 31%, p<0.001). High risk cytogenetics including t(4;14), t(14;16) and del17p were present in 48% of patients in the EM group vs 21% of the others (p<0.001). The percentage of patients included in the highest EM category (EMi: relapsed/refractory disease: 26%; other causes: 6%). Univariate logistic regres-sion analysis demonstrated that ISS, revised ISS (RISS), abnormal LDH, hemoglobin <10g/dl, high risk cytogenetics, and CKD-EPI <40ml/min/1.73m² were independent prognostic factors for EM. In the multivariate analysis ISS and abnormal eGFR were the only independent prognostic factors for EM. When we incorporated ISS and eGFR in a single prognostic model (CKD-EPI/ISS) we identified 3 distinct prognostic groups: 1) low risk group including patients with ISS1 and CKD-EPI <40ml/min/1.73m², 2) high risk group including patients with ISS3 and CKD-EPI <40ml/min/1.73m² and 3) intermediate risk group including patients that did not fit in either low or high risk group. The inci-dence of EM in each was 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: <0.03).

**Summary/Conclusions:** Based on our data, the combination of eGFR esti-mated 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 con-stituting eGFR and ISS are cheap and available for most of MM patients, there-fore the CKD-EPI/ISS prognostic model may be readily applicable. Never-theless, the establishment of CKD-EPI/ISS model requires further validation.

**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 obtained and cocultured with the genetically modified K562-mb15-41BBL cells in order to obtain NKAEs. The activity of NKAE 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-ture media of methylocellulose containing autologous bone marrow and Erythrocytes.

**Results:** SP cells from both cell lines and samples from different stages of MM showed overexpression of stemness markers. Patient NKAE cells were shown to have much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAE cells were able to detect and kill MM cells without significant differences. NKAE cells were also able to destroy CTCs from different MM cell lines. Cyto-toxicity studies revealed that NKAE 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 NKAE cells do not destroy CD34+ clones from healthy bone marrow, confirming the safety of NKAEs.

**Summary/Conclusions:** NKAE cells have molecular characteristics of the tumor stem cell compartment in MM. Likewise, NKAE cells from MM patients could
destroy drug resistant MM cells and clonogenic tumor cells with high efficiency, preserving CD34+ 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 the generalized bone loss in multiple myeloma (MM). Notably, sRANKL may be produced either by proteolytic cleavage of membrane-bound RANKL or by alternative splicing of TNFSF11 gene (TNFSF11 variant 2, sRANKL mRNA). Recent analysis argues against proteolytic processing of the membrane-bound form being the main mechanism of sRANKL production by myeloma cells. Accumulative data indicate that sRANKL mRNA presents a restricted transcriptional pattern, namely is expressed predominantly in malignant cell types. Accordingly, sRANKL mRNA (over)expression in primary MM cells and human MM cell lines has been validated in three independent studies. Furthermore it was recently demonstrated that sRANKL mRNA proximal promoter and 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 TE in the genomic segment comprising TNFSF11, TNFSF11 RNA-Seq data, generated by the GTEx project across 51 normal human tissues, were analyzed via GTeX Portal. TNFSF11 RNA-seq data from 4 bone marrow samples and 8 white blood cells samples, generated from the PRJEB4337 and PRJNA182351 BioProjects, were analyzed via the NCBI portal. TNFSF11 transcription factor (TF) ChIP-seq data were downloaded from the UCSC Genome Browser Database. Data on TNFSF11 proximal promoter methylation status in 63 cell lines were downloaded from the HaIB Methyl450 ENCODE track.

Results: RNA-Seq data from 51 normal human tissues show that sRANKL mRNA is expressed in a cell context-dependent manner, which is in accordance with the retroviral origin of the transcript. Data analysis from the PRJEB4337 and PRJNA182351 BioProjects further validates the null expression of sRANKL mRNA in normal human bone marrow and white blood cells. Methylation status of sRANKL mRNA promoter in 5 lymphoblastoid cell lines (LCLs) signifies that the retroviral promoter remains heavily methylated in these cell types. TNFSF11 TF ChIP-seq data show that 5 of 161 TFs can bind to the TE-derived sRANKL mRNA promoter region. Four of the five TFs (EBF1, PAX5, IKZF1, and PU.1) bind to this genomic segment exclusively in LCLs, signifying a cell-type specific transcriptional regulation. Notably, all 4 TFs are known to play a major role in normal and/or malignant lymphopoesis. 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 development is a direct consequence of 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 a target for new treatment strategies. That Lenalidomide treatment downregulates the amount of sRANKL in the serum of patients with MM through inhibiting PU.1 expression (Breitkreutz et al., Leukemia 2008) is in accordance with the above and further raises the interest on the mechanisms promoting the anti-osteoclastogenic properties of IMiDs.

E1237
ADENOSINE AS THE MYELOMA BONE Marrow Niche: IMMUNE REGULATION 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 inflammation and immune responses. Multiple myeloma (MM), a plasma cell malignancy that develops within the bone marrow (BM) niche, overexpresses CD38, a molecule with complex functions. As a nucleotide-metabolizing enzyme, CD38 catalyzes the initial disassembly of NAD to CADPR and ADPR, which is followed by adenosinergic activity, providing that BM cells are able to recognize the presence of adenosine. (E203a and CD73).

Aims: To demonstrate that adenosinergic pathways contribute to customize hematosis in MM.

Methods: Evaluation of the expression of adenosinergic enzymes was assessed by immunohistochemical and flow cytometric analysis on cell lines, and primary myeloma cells and BM biopsies from patients with MM or with monoclonal gammopathy of undetermined significance. Flow cytometry and immunohistochemistry revealed that 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 a target for new treatment strategies. That Lenalidomide treatment downregulates the amount of sRANKL in the serum of patients with MM through inhibiting PU.1 expression (Breitkreutz et al., Leukemia 2008) is in accordance with the above and further raises the interest on the mechanisms promoting the anti-osteoclastogenic properties of IMiDs.

Summary/Conclusions: We confirm the clinical value of a simple, rapid, two-tubes FC quantification of the proportion of pathologic plasma cells in the evaluation of the risk and kinetics of disease evolution imgUS and SMM. It’s use allows identification of patients which require more frequent follow-up.
Myeloma and other monoclonal gammopathies - Clinical

Results: A total of 1493 pts were enrolled in Cohort 1 from Sep 2009 to Dec 2011; 1450 were treated, 81% (n=1173) in a community setting. Of those, 432 (29%) met analysis criteria. Median follow-up was 39.3 months. Median age was 60 y (range, 24-78); 60% were men; and 86% were white. A total of 165 pts did not receive maintenance. Of 267 pts receiving maintenance, 213 (80%) received LEN-based maintenance; 30 (11%) received BORT-based maintenance; and 16 (6%) received LEN+BORT maintenance. Of the maintenance groups, only data from LEN maintenance is presented; small sample sizes in the other maintenance groups limited interpretation. The median treatment duration was 35.2 months for pts who received LEN maintenance and 26.1 months for those who did not receive maintenance. Median PFS was significantly longer for pts who received LEN maintenance vs no maintenance (50.3 months vs 30.8 months; hazard ratio [HR]=0.62 [95% CI: 0.46, 0.82]; P=0.009; Table). OS was also significantly improved for pts who received LEN maintenance vs no maintenance (HR=0.54 [95% CI: 0.36, 0.83]; P=0.005). Second PFS (PFS for second-line treatment) was similar for both LEN and no maintenance groups. Exploratory analyses showed generally similar PFS and OS outcomes was performed.

Table 1.

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<th>Median PFS (mo)</th>
<th>50%</th>
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<td>LEN</td>
<td>50.3</td>
<td>56</td>
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<td>BORT</td>
<td>30.8</td>
<td>52</td>
<td>42</td>
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<td>LEN+BORT</td>
<td>26.1</td>
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Background: Randomized phase 3 clinical trials have shown that maintenance therapy after autologous stem cell transplant (ASCT) can extend time to progression, progression-free survival (PFS), and overall survival (OS) for patients (pts) with newly diagnosed multiple myeloma (NDMM) (Sonneveld, J Clin Oncol, 2012; McCarthy, N Engl J Med, 2012; Attal, N Engl J Med, 2012; Palumbo, N Engl J Med, 2014; Attal, ASCO, 2016). Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns and outcomes in pts with NDMM in clinical practice.

Aims: The Connect MM registry was used to assess impact of maintenance therapy on survival outcomes in pts with NDMM receiving ASCT. Methods: Adult pts with NDMM were eligible to enroll in the registry within 60 days of diagnosis. Pts were enrolled in 2 sequential cohorts and were treated at the clinician’s discretion as per standard of care. Cohort 1 pts receiving induction and ASCT were included in the analysis and characterized into 4 maintenance regimen subgroups: no maintenance, lenalidomide (LEN)-based maintenance, bortezomib (BORT)-based maintenance, and LEN+BORT maintenance. Duration was from 100 days post-ASCT (no maintenance group) or start of maintenance until progressive disease, death, discontinuation, or data cutoff of January 7, 2016. End points were PFS, second PFS, OS, and safety. An exploratory analysis of the impact of baseline characteristics on survival outcomes was performed.
E1240

DARATUMUMAB-BASED COMBINATION THERAPIES IN HEAVILY-PRE-TREATED PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA

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Background: Daratumumab-based combination Therapies (DCT) with bortezomib (V)/ lenalidomide (R)/ pomalidomide (P) and dexamethasone (d) have shown exceptional activity in relapsed and/or refractory multiple myeloma (RRMM) in trials. Experience outside of trials since the approval of Daratumumab (D) in 2015 is limited.

Aims: We aimed to review the outcomes of patients who received DCT at our institution.

Methods: Records of RRMM patients seen at Mayo Clinic, MN from December 2015–December 2016 were reviewed. Patients who received ≥1 cycle of DCT were included. Time-to-event analyses were done from date of starting DCT using Kaplan Meier method. Common terminology criteria for adverse events vs

Results: Of 130 patients, 59% were males and median age at DCT initiation was 67 (43-93) years. ECOG performance score was 22% 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%) patients received ‘other’ DCT. Median time to first response (≥ PR) was 3.1 months (95% CI: 17-4.6). Overall response rate was 46%, complete remission in 17%, with clinical benefit rate of 62%. Median estimated follow up from initiation of DCT was 5.4 months (CI 4.2-5.5). The median duration of response was 6.1 months (CI 5.1- not reached (NR)). Median progression-free survival (PFS) was 5.5 months (CI 4.1-7.8) (figure A) and median time to next therapy (TTNT) was 5.9 months (CI 4.6-9.4) (figure B). Median PFS for D/Pd, D/Rd and other DCTs were 4.6 (CI 2.7-NR), 7.8 (CI 5-NR), 3.9 (CI 2.1-4.8) and 3.9 (CI 2.8-8.2) months, respectively (p=0.3). Median overall survival (OS) from starting DCT was NR (CI 11.4-NR) (figure C). Median PFS for quadruple refractory (n=28) MM was 2.8 months (CI 2.2-5.3) vs 5.9 months (CI 4.9-NR) for the rest (p=0.008) (figure D). Grade 3 or higher hematological toxicities were seen in 42% of patients. Other toxicities included infections (37%), fatigue (31%), infusion reactions (16%) and diarrhea (10%).

Figure 1.

Summary/Conclusions: DCT are effective in RRMM, but the PFS remains short, particularly in quadruple refractory patients, reflecting the challenges encountered in managing heavily-petreated, and often less fit patients, in routine practice.

E1241

IMPACT OF METFORMIN USE IN THE OUTCOMES OF MULTIPLE MYELOMA PATIENTS POST STEM CELL TRANSPLANT

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Background: 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.

Methods: All MM patients who underwent stem cell transplant (SCT) at the Mayo Clinic Rochester from 2007 to 2012 were reviewed. Patients were grouped based on metformin use. Initial diagnosis at our institution and ≥12 months of follow up were required. Kaplan-Meier method and Cox regression were used for time-to-event and multivariate analysis.

Results: Out of 687 patients, 78 (11.4%) patients were using metformin at the time of MM diagnosis. Baseline characteristics in the metformin and no-metformin groups were similar. Median metformin dose was 2000mg daily and median duration of metformin use from MM diagnosis was 22 months. Patients on the Metformin group achieved higher rates of complete response after SCT (41% vs 29% p<0.02). Median progression-free survival (PFS) after SCT was longer in the Metformin group, 31.3 months (95% CI: 10.4-52.2) vs 16.6 months in the no-metformin group (95%CI: 14.5-18.7) p<0.04. There was a trend toward longer overall survival in the Metformin group, but it was not statistically significant (170 vs 106 months, p<0.10). In a multivariate analysis of metformin use, age, sex, international staging system (ISS), LDH and cytogenetics/FISH, the former was an independent predictor of PFS after SCT (OR: 0.38, 95%CI: 0.20-0.88, p<0.001).

Figure 1.

Summary/Conclusions: Metformin use was associated with a better progression-free survival and higher complete response rates after SCT in our MM cohort. A trend toward better overall survival was also noted in the Metformin group. Larger studies are needed to enhance our understanding of the 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 of myeloma. Whole body MRI (WBMRI) has shown exceptional activity in relapsed and/or refractory multiple myeloma. Whole body PET-CT (WBCT) is a well-established imaging modality in myeloma. The aim of this study was to compare WBMRI with PET-CT imaging at diagnosis of myeloma.

Methods: A retrospective cohort study was performed of all patients with multiple myeloma or plasmacytoma in 33 patients presenting to King’s College Hospital, London. The scans were reviewed independently by two consultants in Radiology.
and Nuclear Medicine, looking for focal bone lesions, bone marrow pattern and incidental findings. Details of the patients’ demographics, myeloma diagnosis and treatment were collected from the medical records.

**Results:** Of the 33 patients, 24 were male. The median age was 64 years (range=43-86 years). One patient had a solitary plasmacytoma, the other 32 had myeloma (21 IgG, 3 IgA, 2 non-secretory, 4 light chain disease, 2 biclonal myeloma, 1 had ISS stage 1 disease with a median paraprotein level 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 difference was in the detection ofvertebrae L5 (using diffusion weighted MR imaging) was measured. This showed a strong correlation with the degree of bone marrow infiltration by plasma cells (r=0.64). An ADC of <600mm²/s had a negative predictive value of 93% for a bone marrow plasma cell infiltrate of >60%. There was also a significant difference (p=0.012) in the ADC between those with smouldering myeloma and those with symptomatic disease. It was noted that 9 scans resulted in incidental findings including pneumonia, adrenal lesions and one case of colocoleral cancer.

**Summary/Conclusions:** We have shown no difference in PET-CT and WBMRI in detecting a myeloma defining focal bone lesion, or providing prognostic estimations of burden of disease. Using MRI, a measure of the ADC at vertebrab 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.

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**PERSISTENCE OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY CAN HINDER RECOVERY OF ORGAN DAMAGE IN PATIENTS WITH AL AMYLOIDOSIS**

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**Background:** In multiple myeloma, Minimal Residual Disease (MRD) demonstrated by multiparameter flow cytometry (MFC) identifies subjects with significantly shorter survival typically among those who attain complete response (CR). The role of MRD in AL amyloidosis has not been assessed so far.

**Aims:** In the present study, we assessed the MRD by MFC in patients with AL amyloidosis who attained CR.

**Methods:** CR was defined as per current criteria (negative serum and urine immunofixation and normal free light chain ratio). For flow cytometry studies bone marrow samples were processed following the Euro Flow Bulk Lysis Standard Operating Protocol and stained with the EuroFlow/MF MM MRD panel. At least 5x10⁶ events were measured using a FACS Cantos II (USA) instrument. Data were analyzed using the Infinty 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 were 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 relapsed and 9 (45%) progressed by detection of smouldering disease. More than 2 lines of therapy were required to achieve CR in 7 subjects. Median time to CR was 10 months (range: 3-82). Five patients (62%) had achieved cardiac response and 9 (50%) renal response at the time of CR. The median time from CR to MRD was 30 months (range: 6-148), this was not different in the MRD positive and MRD- patients. 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 difference was in the detection of vertebrab L5 (using diffusion weighted MR imaging) was measured. This showed a strong correlation with the degree of bone marrow infiltration by plasma cells (r=0.64). An ADC of <600mm²/s had a negative predictive value of 93% for a bone marrow plasma cell infiltrate of >60%. There was also a significant difference (p=0.012) in the ADC between those with smouldering myeloma and those with symptomatic disease. It was noted that 9 scans resulted in incidental findings including pneumonia, adrenal lesions and one case of colorectal cancer.

**Summary/Conclusions:** We have shown no difference in PET-CT and WBMRI in detecting a myeloma defining focal bone lesion, or providing prognostic estimations of burden of disease. Using MRI, a measure of the ADC at vertebrab 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.

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**ERRATUM**

**ERRATUM**

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**A validation study in a larger cohort is ongoing.**

**Further investigations are needed in the area of whole body imaging.**

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**Aims:** Carfilzomib (K), a novel irreversible proteasome inhibitor associated with a low incidence of PN, was evaluated in two recent phase 3 studies in RRMM patients. The possible impact of MRD on PN rate during the study (prespecified key secondary endpoint) was significantly lower with Kd vs Rd for patients with grade 2 PN at baseline. In ENDEAVOR, grade ≥2 PN rate during the study (prespecified key secondary endpoint) was significantly lower with Kd vs Vd (6.0% vs 32.0%, Table). Patients had significantly improved pain and neurotoxicity subscale scores with Kd vs Vd. PFS improved with Kd vs Vd in patients with baseline history of grade 2 PN (Table 1).

**Table 1.**

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**Summary/Conclusions:** In ENDEAVOR, the rate of PN was significantly lower with Kd then with Vd: in ASPIRE, PN rate was similar for Kd and Rd. Improved pain and neurotoxicity subscale scores with K may be attributed to better disease control and/or lower PN rates.

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**EARLY RELAPSE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA IS A POOR PROGNOSTIC MARKER FOR OVERALL SURVIVAL AND IS DIFFICULT TO PREDICT AT DIAGNOSIS OR DURING INDUCTION TREATMENT**

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**Background:** High dose chemotherapy followed by autologous stem cell transplantation (ASCT) remains the gold standard treatment in myeloma for young
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 $\geq 50 \times 10^9/l$ and platelets $\geq 20 \times 10^9/l$. 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 transplant was immunomodulatory drug (IMiD) based (THAL/LEN) for 318/443 patients & 72/443 were proteasome inhibitor (PI) based (BORT/CAF). 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 $<12$m or $\geq 12$m 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. Median OS post ASCT was 31 months (95% CI 21-39) compared to non-progressive patients (median OS:103m 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:89m (95% CI 79-98m) $p=0.0005$. 1st line therapy did not influence likelihood of PFS<$\leq$12months, with no statistical difference between patients who received PIs, IMiDs or both 1st line ($p=0.484$). A significant difference was observed in median time to platelet engraftment between the 2 centres. Increased time for platelets to reach $>20 \times 10^9/l$ 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 somoral factors which facilitate disease resistance and impair normal haematopoiesis. All of these factors have been shown to drive relapse. RCTs 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 this extremely poor prognosis group.

**E1246**

**PATIENT-REPORTED OUTCOMES (PROS) WITH IBRUTINIB: SUBSTUDY OF INNOVATETM FOR WALDENSTRÖM 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 WM therapy. Ibrutinib (ibr), a first-in-class, once-daily inhibitor of BTK, is indicated in the EU for the treatment of WM after 1st prior therapy of first-line in patients (pts) unsuitable for chemoimmunotherapy.

**Aims:** To prospectively collect PROs from the INNOVATE substudy to assess patients’ perspectives of the therapeutic benefit of ibr.

**Methods:** INNOVATE has randomized (ibr+rituximab [RTX] vs placebo+RTX) with 2 components. Pts in the substudy received oral ibr 420mg daily until progressive disease (PD) or unacceptable toxicity. All pts provided informed consent. PRO assessments—FACT-An total score (TS) and FACT-An anemia subscale (AS), and EQ-5D-3L (EQ)—were performed regularly.

**Results:** Persistent fatigue was the main indication for treatment in 22/31 (71%) pts. Baseline PRO scores were lower for time study vs randomized pts (Table). With a median of 17 months (mo) of treatment, most pts had clinically meaningful improvement in TS (>7 points; 77%), AS (>6 points; 84%), and EQ utility scores (≥0.08 points; 68%). Time to clinically meaningful improvement was prompt (1 mo for TS and AS; 2 mo for EQ), corresponding with a 48% decline in median IgM (median 20 g/L) after 4 weeks. In pts with baseline anemia (hemoglobin [Hb] ≤110 g/L), sustained Hb improvement increased with depth of response. At week 65, Hb levels significantly correlated with TS ($r=0.507$, $P=0.01$) and AS ($r=0.519$, $P=0.008$), and were marginal for EQ ($r=0.39$, $P=0.054$). Although IgM levels did not significantly correlate with PRO scores, the benefit was similar in responders regardless of depth of response.

**Table 1.**

**Summary/Conclusions:** Clinical response, and associated anemia improvement induced by ibr, correlated with meaningful improvements in the well-being of heavily pretreated pts with RTX-refractory WM.

**E1247**

**INCIDENCE AND RISK FACTORS OF CARDIOVASCULAR ADVERSE EVENTS IN A LARGE POPULATION OF NEWLY-DIAGNOSED, TRANSPANT 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:** To prospectively collect CV outcomes in pts newly-diagnosed with MM treated with Carfilzomib in 3 phase III studies (IST-CAR-506, IST-CAR-508, IST-CAR-601).

**Methods:** All pts were treated with 9, 28-day induction cycles with carfilzomib, cyclophosphamide (300mg/m² on days 1,8,15) and dexamethasone (40mg weekly) (CCyd), followed by carfilzomib maintenance until progression or intel-
eral 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 cardiovascular (CV) AEs occurred in 29% of patients; any grade hypertension was 34% of patients and those with at least 1 CV risk factor were at higher risk (odds ratio: 3.75; p=0.002) and grade 3-5 CV-AEs (34% vs 15%, p=0.01) were more frequent compared to those without CV-AEs (12% and 18%, respectively; p<0.001). A trend toward a shorter 2-year overall survival (adjusted for age) was observed among patients who experienced at least 1 CV-AE as compared with those who did not (74% vs 83%; HR: 0.51; p=0.066). Pts ≥75 years had a higher risk of any grade (58% vs 36%; p=0.002) and grade 3-5 CV-AEs (34% vs 15%, p=0.01) were more frequent compared to those younger 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.

Summary/Conclusions: Among newly diagnosed MM pts treated with carfilzomib, cyclophosphamide and dexamethasone, at least 1 CV-affected event was more common in pts aged ≥75 years than in younger patients (8% vs 13%; HR: 0.67, p=0.05). CV toxicity may significantly impact on treatment compliance and survival. Therefore, to derive maximum benefit from Carfilzomib, all pts – particularly the elderly - should be carefully assessed to select the most appropriate treatment.

E1249 POMALIDOMIDE (POM) + LOW-DOSE DEXAMETHASONE (LODEX) AFTER SECOND-LINE LENALIDOMIDE (LEN)-BASED TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED PROGRESSION-FREE SURVIVAL ANALYSIS D.S. Siegel1, G.J. Schiller2, K. Song3, R. Agajanian2, K. Stockett-Goldstein6, H. Kaye4, M. Selagi2, F.J. Rivest2, E. Malek2, G. Talamaro1, J. Mouru1, W. Chung1, S. Sinnivasan1, M. Qian11, S. Rivulski1, A. Thakurta11, N.J. Bals12 1John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, NJ, 2David Geffen School of Medicine at UCLA, Los Angeles, CA, 3United States, 4Virginia General Hospital, Vancouver, BC, Canada, 5The Oncology Institute of Hope and Innovation, Bronx, NY, 6McGill University Health Centre, Montreal, Quebec, Canada, 7Cleveland Clinic, 8University Hospitals Case Medical Center, Cleveland, OH, 9Penn State Hershey Cancer Institute, Hershey, PA, 10Cleveland Cancer Institute, Summit, NJ, 11United States, 12University of Calgary, Calgary, Alberta, Canada

Background: Most recent pivotal trials of triple therapy in second- and third-line treatment excluded patients (pts) whose multiple myeloma (MM) was refractory to LEN. This is not reflective of the standard of care in first and second line where LEN is given until progressive disease (PD). To address this, the MM-014 phase 2 trial enrolled pts with RRMM and second-line LEN-based treatment failure. Cohort A enrolled pts treated with POM + LoDEX. The study was amended to include cohort B (pts treated with POM + LoDEX + daratumumab).

Aims: To present updated safety and efficacy analyses only from cohort A, in which pts received POM + LoDEX immediately after relapsing or being refractory to second-line LEN-based therapy.

Methods: Pts aged ≥18 years had documented MM, measurable disease, 2 prior lines of treatment, and PD after ≥2 cycles of second-line LEN-based treatment. Pts received 28-day cycles of POM 4mg/day on days 1-21 + LoDEX 40mg/daily (1day/75 days) on days 1, 8, 15, 29 and 36; R=42% (cortenbrolins) was mandatory. The primary endpoint was overall response rate (ORR; ≥ partial response [PR]) assessed by modified IMWG criteria. Secondary endpoints included time to response (TRR), progression-free survival (PFS), secondary primary malignancies (SPMs), and biomarkers. All pts provided informed consent.

Results: Of 51 enrolled pts in cohort A, 59 (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 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 ≥ TRR (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 [9.8%]. No pts experienced SPMs. In the immune subset analysis, the proportions of CD3+ and CD3+/CD8+ T cells after treatment were significantly higher vs baseline (72.6% vs 67.8% and 36.9% vs 32.1%, respectively, P<0.05). Pts with response also had significantly elevated proportions of these T-cell populations, but pts with no response did not. Relative changes from baseline for CD3+ and CD3+/CD8+ T-cell populations were significantly greater in pts with response vs those with no response (10.4 vs −0.8 and 4.2 vs −3.5, respectively; P<0.05).

Table 1

<table>
<thead>
<tr>
<th>POM Treatment Duration, months</th>
<th>≥ PR (n=15)</th>
<th>≥ MR (n=27)</th>
<th>≥ PR (n=15)</th>
<th>≥ MR (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable (n=1)</td>
<td>7 (47%)</td>
<td>7 (26%)</td>
<td>7 (47%)</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>Evaluated (n=29)</td>
<td>15 (52%)</td>
<td>12 (41%)</td>
<td>7 (23%)</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>Progressed (n=2)</td>
<td>4 (67%)</td>
<td>4 (50%)</td>
<td>1 (14%)</td>
<td>1 (14%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This update confirms the safety and efficacy of POM + LoDEX following second-line LEN-based treatment failure in pts with RRMM. Hematologic AE rates improved, and median PFS was longer with third-line use than previously reported with POM + LoDEX use in later treatment lines. In addition, achieving disease control of ≥ MR led to similar PFS rates as reaching ≥ PR.

E1249 “REAL WORLD” DATA ON THE EFFICACY AND SAFETY OF IZAXOMIB IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A STUDY OF THE GREEK MYELOMA STUDY GROUP

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Background: The all-oral combination of ixazomib, lenalidomide and dexamethasone (IRd) has been recently approved as a novel standard of care for relapsed/refractory multiple myeloma (RRMM). Aims: 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 safety and efficacy; 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 (83%, median age 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 those who were on treatment. 63 were treated with LEN, 6 with BORT, 0 with THAL, and 4.6 mos (range 0.2-36.9 mos) for THAL. At the time of analysis, 3557 patients (98.0%) had discontinued treatment. Of the 73 patients (2.0%) for BORT, and 4.6 mos (range 0.1-79.9 mos) for patients receiving LEN, 4.1 mos (range 0-61.4 mos) for patients receiving THAL. Data on long-term responders will be presented at this meeting.

Table 1.

Summary/Conclusions: LEN was generally well tolerated and the safety results were similar to published data. As expected, the occurrence of neuropenia, 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.
Results: During the development process, a number of similarities and discrepancies between centers as well as evidence gaps were identified. Intense discussion and literature searches resulted in a concise, harmonized clinical pathway, released by all 14 Centers of Excellence. This is freely available on the website ccc-netzwerk.de and provides a very decisive insight according to the current state of knowledge on the CCC-level (e.g. on the diagnostic algorithm). The clinical pathway is well suited for informing patients and physicians about the most up-to-date, comprehensive medical treatment standards as well as innovative procedures. Furthermore, this project initiated the idea of developing a national evidence-based clinical practice guideline for MM in the frame of the German Guideline Program in Oncology.

E1252
WT1 HETEROCLITIC EPITOPPE IMMUNIZATION FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MULTIPLE MYELOMA (MM)
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Background: The Wilms tumor 1 (WT1) protein is a tumor associated antigen that is a target for anticancer immunotherapy. We had previously demonstrated overexpression of WT1 in multiple myeloma (MM) cells by IHC, as well as formation of a WT1 peptide fragment (RMPFNPYLY-LHxA-01) on MM cells using the engagement interface between malignant plasma cells and T-cells in HL-A-A’1*11 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 galinepimut-S (GPS) after autologous stem cell transplantation.

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 autologous stem cell transplantation with melphalan conditioning followed by (fth) 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]. GPS was administered with montanide s.c. starting autologous stem cell transplantation.

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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 guidelines will increase the quality of care throughout Germany. To be up-to-date and to reflect latest research findings this clinical pathway will be updated every 18 months.

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. Rosirol 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; Pirris 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 analyze the impact of PD on OS. Our research included the influence of PD on OS and the survival of patients with at least PD at time of ASCT (non-PD patients).

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 impact on their response before 1st ASCT (d -100 or, if available, response as close to the date of ASCT as possible, i.e. until d-4). Of the 874 ASCT-patients, 829 were eligible for a PFS- and 832 for an OS-analysis. In 51 patients, PD was present at time of ASCT. PFS and OS of those patients were compared with the survival of patients with at least SD at time of ASCT (non-PD patients).

Results: Neither the clinical parameters at induction start; Parrish et al. (2015) stated a significantly shorter PFS, but not OS, in patients with PD compared to SD at time of ASCT. Furthermore, clinical factors at beginning of induction therapy, including age (< 65 years), ISS stage, elevated LDH, use of novel agents in induction therapy, high-risk FISH cytogenetics (at least one of the following: del17p, 1q21 gain, t(14;16)); response after ASCT, and maintenance therapy (yes vs no) were analyzed regarding their impact on PFS and OS of patients transplanted in PD. We also analyzed clinical factors at beginning of PD as well as use of novel agents in the induction therapy regarding their impact on the survival of PD before ASCT. Response was evaluated according to EBMT-criteria. PFS was calculated from date of 1st ASCT, except for prognostic impact of response assessment after 1st ASCT, where date of response assessment was used. Start of maintenance therapy was analyzed as time-dependent factor.

Summary/Conclusions: Non-trial patients transplanted in our center between 1992 and 2014 with ≥ 100 days before ASCT had similar PFS and OS as non-PD patients. Neither the clinical parameters at induction start, response after 1st ASCT, nor maintenance therapy had a significant effect on PFS in those patients. In the univariate analysis, high-risk cytogenetics as well as elevated LDH at induction start had a significantly negative effect on OS in patients with PD before ASCT (HR= 17.12, p = 0.0017; HR= 6.09, p = 0.01, respectively), compared to PD-patients with no high-risk cytogenetics or with normal LDH. Furthermore, ISS stage III was a significant predictor (OR= 3.35, p = 0.02)) of occurrence of PD before ASCT.

E1254
SEVERE INFECTIONS IMPACTS OVERALL SURVIVAL IN ACTIVE MULTIPLE MYELOMA PATIENTS
G. Barilla1,*, C. Nicolo1, A. Lipo1, A. Brancha1, L. Checuz1, E. De March1, Madrid, Spain, June 22 – 25, 2017

haematologica | 2017; 102(s2) | S13

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 guidelines will increase the quality of care throughout Germany. To be up-to-date and to reflect latest research findings this clinical pathway will be updated every 18 months.
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 test if 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 (baseline). 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 66.4 ± 11.4 years). A review of MM comorbidities showed that both proteasome inhibitors (PIs) and immunomodulatory drugs (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. Given that severe comorbidities impact OS mostly in the setting of not neutropenia related infections, immunoglobulin replacement therapy or chemotherapy 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.

Figure 1.

Summary/Conclusions: PI+IMiDs may be associated with incremental occurrence of specific CV events during treatment, and may result in specific CV events earlier during therapy than PIs or IMiDs alone. These highlight a need for treatments that do not exacerbate CV risks and are appropriate for patients with pre-existing CV conditions. The lower prevalence of baseline CV comorbidities and lower mean age in patients on PI+IMiD 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 combined with low dose dexamethasone (LD), more recent studies have used lenalidomide plus low-dose dexamethasone (LD) for relapsed/refractory MM (RRMM), as the LD regimen demonstrated better survival with lower toxicity for the treatment of newly diagnosed MM.
Methods: We collected the clinical data of 169 patients qualified to HDT/autoSCT in routine clinical practice.

Aims: We performed a historical comparison based on a systematic review of literature describing low- vs high-dose dexamethasone in patients with MM to assess effects of LD vs LD on safety and efficacy outcomes.

Methods: We searched MEDLINE, EMBASE and Cochrane databases and key clinical trial registries for studies including adults with MMR who had received ≥3 prior therapies and had a symptomatic relapse on their last treatment.

Eligible studies evaluated LD (lenalidomide: 25mg on Day 1–21 of each cycle; dexamethasone: 160mg/cycle, not pulsated) or LD (Cycles 1–4: 480mg/cycle; Cycle 5+; 160mg/cycle, pulsed). Only those trials with designs and baseline patient characteristics similar to ELOQUENT-2 were eligible to ELOQUENT-2 to enhance reliability.

Studies with a follow-up of ≥16–25 months were evaluated separately from studies with a follow-up of >30 months; these observation periods approximately align with those of ELOQUENT-2.

Results: From an initial bibliographic search yielding 5155 non-duplicate results and 619 registry results, 7 studies (8 publications) met the inclusion criteria (4 LD studies, 3 LD studies). Data for overall survival and tolerability from 1153 patients in the LD group and 353 patients in the LD group were analyzed. The median age of patients was 63–88 years. Most patients were white, male and had an ECOG score ≤ 1. LD was not associated with loss of efficacy in terms of overall survival; after >30 months of follow-up, the hazard ratio for LD vs LD was 1.04 (95% CI 0.85–1.28). Tolerability was similar for LD vs LD; after 16–25 months of follow-up, LD was associated with a statistically significantly increased risk of Grade 3/4 adverse events (AEs; relative risk [RR]: 1.10 [95% CI 1.01-1.18]). However, after >30 months of follow-up, LD was not associated with an increased risk of Grade 3/4 AEs (RR: 1.05 [95% CI 0.97-1.12]) or serious AEs (RR: 1.08 [95% CI 0.97-1.20]); RR for AEs leading to discontinuation was 1.16 (95% CI 0.87-1.54).

Summary/Conclusions: Overall survival and safety are not significantly affected by different dosing of dexamethasone in combination with lenalidomide; thus, using LD is a reasonable alternative for patients in this patient population. Further studies may provide additional evidence to inform clinicians and revision of international guidelines for dexamethasone dosing in MM.

E1257

HIGH EFFICACY AND SAFETY OF VTD AS AN INDUCTION PROTOCOL IN NEWLY DIAGNOSED MM PATIENTS ELIGIBLE FOR HD/autOSCT – A REPORT OF POLISH MULTIPLE MYELOMA STUDY GROUP


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Background: Three drug bortezomib-based regimens are nowadays generally recommended standard induction therapy for transplant-eligible patients with newly diagnosed multiple myeloma (MM). The choice between different regimens depends on drug availability in particular countries, their toxicity profile and local preferences. Observations from routine practice might have though significant impact on the MM management in this patient population.

Aims: The aim of this retrospective analysis was to evaluate the efficacy and safety of VTD regimen in newly diagnosed MM patients eligible for HD/autOSCT in routine clinical practice.

Methods: We collected the clinical data of 169 patients qualified to HD/autOSCT who received VTD as induction, 105 patients (62%) received VTD as induction and 105 patients (62%) received VTD as induction. The patients were 12 men and 7 women, aged 60±4 years (37–73 years). 10 patients were diagnosed with lambda FLC MM and 9 with kappa type. A total of 244 sessions were conducted, with an average of 11.6 sessions per patient (range 3-27). In all cases reduction of serum FLCs concentration was successfully achieved. (90% of reduction). At the end of treatment with HCO-HD, the reduction of lambda and kappa FLCs concentrations was 85% (range 30-98%) and 85% (range 30-98%), respectively. The average reduction per dialysis session was 65% for lambda and 60% for kappa. 17 out of the 21 treated cases recovered sufficient renal function to become independent of dialysis (80.9% renal recovery). Renal recovery appears to be sustained over time. There was a significant association between the reduction of lambda and kappa FLCs concentrations and renal recovery. Our results confirm previous findings on the effectiveness of FLCs reduction by HCO-HD. Until randomized trials yield results, our highest percentage of improve renal outcome with respect to published studies leads us to recommend combined therapy of chemotherapy and HCO-HD in patients with MM-related renal failure.

Summary/Conclusions: In dialysis-dependent AKI secondary to MM, combination HCO HD with chemotherapy allows a sustained reduction of FLCs levels, representing an effective therapy in renal recovery.
E1259
IMPACT OF IMMUNOPARESIS IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS
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Background: The presence of immunoparesis (IP) at diagnosis in several plasma cell disorders is a risk factor for progression, associated with an unfavorable outcome with reduced progression-free survival (PFS) and overall survival (OS). However, its impact in light chain (AL) amyloidosis has been evaluated only in few series, and when present it was associated with worst response and survival.

Aims: The aim of this study was to investigate the prognostic impact of IP in patients with newly diagnosed AL amyloidosis at a single institution.

Methods: We reviewed the clinical records of patients with AL amyloidosis diagnosed from January 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 (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 54.3%. 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. 21.7%; 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=0.015). The presence of IP was an independent risk factor for worse PFS and OS in multivariate analysis. PFS and OS were significantly different in patients with stage I and II Mayo12, incorporating ASCT, BMPC and IP, indicating that IP retained its independent prognostic factor for worse PFS (HR=12.05; 95% CI, 1.9-75.7; P=0.008).

Discussion: 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.

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.

E1260
TREATMENT PATTERNS AND DURATION OF TREATMENT IN JAPANESE MULTIPLE MYELOMA PATIENTS RECEIVING SECOND LINE THERAPY WITH NOVEL AGENTS
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Background: The introduction of novel agents, such as proteasome inhibitors (PSIs) and immunomodulatory drugs (IMiDs) approved in 2006 and 2010, respectively, and/or autologous stem cell transplantation (SCT) are associated with improved overall survival of 60.6 months in Japanese multiple myeloma (MM) patients (pts) (Ozaki et al. Blood Cancer Journal 2015). However, the disease still remains incurable with disease relapse being inevitable after frontline therapy (FLT). Data regarding treatment patterns and duration of treatment (DOT) of Japanese pts with relapsed and refractory (RR) MM in routine clinical practice is limited.

Aims: This retrospective study aims to describe the treatment patterns and DOT of second-line therapy (SLT) with PI- and IMiD-based regimens and to assess factors that influence treatment choice and DOT of SLT in Japanese MM pts.

Methods: This is retrospective cohort study in pts with MM diagnoses with ICD-10-CM (C900) codes between April 2008 and January 2016 in Japan. This study used Japanese health insurance data provided by Medical Data Vision. MM pts receiving SLT were included. Index date was defined as the first observed claim for MM treatment and SLT was defined as switch to another drug combination or retreatment following a treatment gap of 90 days after starting FLT. Pts with salvage SCT were excluded. Observations were censored at loss to follow up, death or the end of study period. Kaplan-Meier analyses were performed to calculate DOT from the start of SLT. Welch’s test was used to test for statistical significance between groups.

Results: Among 965 pts receiving SLT, mean age was 68.8 years of age (yo); 65.3% were ≥65 yo at start of SLT; 54.2% were male. Most pts received lenalidomide (L)-based SLT (35.4%), followed by bortezomib (B)-based regimens (29.4%) and other regimens not containing novel agents (35.2%); Other regimens include thalidomide, cyclophosphamide, etoposide, melphalan, vincristine, (liposomal) doxorubicin, interferon, panobinostat, single-agent steroid; only 1.2% received B+L combination therapy. L+d and B+d were the most common (35.2% vs. 21.7%) in SLT. Majority of Japanese pts received B-based regimen in FLT among those receiving L+d and B+d SLT (77.2% vs. 55.1%). Pts with peripheral neuropathy (PN) and renal insufficiency (RI) prior to SLT were 30.3% and 16.5%, respectively; those with PN were more likely to receive L+d compared to B+d (35.9% vs 21.3%, P=0.0047), but those with RI was not independently associated with treatment choice of SLT. Median DOT of L+d was longer than B+d (13.8 vs 6.9 months, P=0.0001); DOT was similar for those without a front-line SCT and receiving B+d FLT in both regimens (11.9 vs 11.9 months). PN and RI prior to SLT and age have not shortened the DOT in SLT. Additionally, 35.4% experienced PN during SLT among pts receiving L+d and B+d in SLT but there was no significant statistical difference of DOT between pts with and without PN. Median daily dose of L was 12.0mg; there was no significant difference of DOT between pts received at least and less than 12.0mg.

Summary/Conclusions: Among pts in SLT, 65% of Japanese pts obtained L- and B-based regimens. This observation is similar to the United States (Romanus et al. EHA 2016) and Europe (Raab et al. EHA 2015). Majority of pts did not receive triplet-based regimen. Pts experienced PN in FLT were more likely to initiate L-based therapy in SLT and regimen type in SLT was correlated with DOT. Future research is needed to better understand treatment changes in routine clinical practice and the impact on pts’ outcomes, especially, after integration of novel agent-based triplet combinations as new standards of care in RRMM in Japan.

References

E1261
ROLE OF HEAVY/LIGHT CHAIN RATIO IN MYELOMA PATIENTS ACHIEVING COMPLETE RESPONSE AFTER FIRST LINE THERAPY
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Background: The presence of immunoparesis (IP) at diagnosis could be an additional powerful discriminatory prognostic indicator in the group of patients with 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). Aims: We conducted a single center, prospective study of HLC ratio, in comparison with free light chain (FLC) ratio, for the evaluation of MRD and its prognostic role in MM patients achieving CR after first line treatments including novel agents.

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 52-80) and progression-free survival (PFS) was 26 months (95% CI 10-38). FLC assay normalisation in CR was significantly associated with better PFS (43 months, 95% CI 14-45) and fourteen samples (56%) with abnormal FLC ratio. Discrepancies and k/λ ratios were then calculated.

HLC ratio and fourteen samples (56%) with abnormal FLC ratio. Discrepancies 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. Discrepancies between the two assays occurred in 11 patients. FLC assay normalisation in CR was significantly associated with better PFS (43 months, 95% CI 14-45) respect to patients with persistent abnormal FLC ratio (12 months, 95% CI 9-22, p=0.049). In contrast, normalization of HLC ratio had no impact on PFS (26 months, 95% CI 52-80) and progression-free survival (PFS) was 26 months (95% CI 10-38).

Aims: 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.

Summary/Conclusions: In this first real-world analysis we confirm that daratumumab monotherapy is able to induce response in one third of highly pretreated and double refractory RRMM patients. Regarding safety, in contrast to the SIRIUS trial where no treatment discontinuations due to AEs occurred, 3/26 pts (11%) 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

Summary/Conclusions: In this first real-world analysis we confirm that daratumumab monotherapy is able to induce response in one third of highly pretreated and double refractory RRMM patients. Regarding safety, in contrast to the SIRIUS trial where no treatment discontinuations due to AEs occurred, 3/26 pts (11%) treated with daratumumab in clinical practice had their therapy interrupted due to complications.
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 cancers1. The medical management of multiple myeloma has changed over the years and is influenced by multiple factors (e.g., evidence from clinical trials, drug approval status, level of drug reimbursement, guidelines), which vary across Europe. Information describing how patients are managed in the real world is needed. Aims: The aim of this analysis was to investigate real-world treatment patterns and patient characteristics in MM across Europe.

Methods: Physicians in Europe were requested to answer a series of questions on patient characteristics and treatment regimens of the last eight patients that they had treated during the month prior to answering the questionnaire, according to their patients’ medical charts. The questionnaire was conducted between January and June 2016. Data on 2564 patients with MM were available and are presented here. Countries were grouped into regions according to similar healthcare systems: Spain, Portugal, Italy and Israel (Southern Region, SR, n=1096); Austria, Netherlands, 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 eligible for autologous stem cell transplant (ASCT) (53-59%). The median time from MM diagnosis to the time that the physician answered the questionnaire was higher in ER (19.9 months) than other regions (9.7-11 months) (Table). The majority of frontline regimens contained bortezomib, although this was lower in ER (51%) than in other regions (66-70%). The median duration of frontline therapy was longer in ER (4.5 months) than other regions (3.2 months). This difference was mainly driven by ASCT eligible patients having longer duration of therapy in ER (4.5 months) than other regions (2-3 months). The number of patients who were treated with ASCT was higher in SR and CNR (24-25% of patients) 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, for 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 (79-87%). Differences in treatment duration were noted between regions with the exception of SR where pomalidomide (29.4%), lenalidomide (12.6%) and bortezomib (14%) were 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.

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, as diagnosis in the form of a classic kidney lesion is rare, but at present the prognosis of chronic renal failure in this context is unknown.

Aims: The study was interested in the prognosis of AL amyloidosis associated with endstage renal disease on dialysis in the era of treatment with 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

E1264

FRAILTY AND MORTALITY IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background: Worldwide, life expectancy continues to rise. The treatment of elderly people with cancer poses special challenges that should be better addressed. Frailty is a geriatric syndrome associated with reduced functional reserve, impairment in multiple physiological systems, and reduced ability to regain physiological homeostasis.

Aims: To evaluate the impact of the level of frailty on early death and overall survival of elderly patients with multiple myeloma.

Methods: Retrospective study of a cohort of 150 patients older than 65 years with a recent diagnosis of multiple myeloma from January 2006 to December 2012. Patients were treated with IMIDs, alkylating or bortezomib based chemotherapy based on physician preference blind to the geriatric assessment. A check list for frailty burden measurement was used based on Edmonton frailty score and included: cognitive impairment, depressive disorder, polypharmacy, urinary incontinence, functional impairment, gait disturbance or falls, low weight or weight loss and previous hospitalization. Level of frailty was scored as the sum of each area involved. Record of all the variables were obtained from a retrospective review of the centralized and computerized medical records of the patients presented with Myeloma ISS 3. Sixty five percent of patients met at least one frailty criteria and 31% of patients were considered frail. The most common findings were polypharmacy, gait and functional impairment. Most patients were treated with IMIDs (47%); alkylating agents (33%) or bortezomib (14%) based chemotherapy. There was no difference in treatment according to frailty group (p=0.38). The median overall survival time was 75 months (95% CI 53-110), 39 months (95% CI 19-64) and 17 months (95% CI 5-37) for fit, vulnerable and frail patients respectively (log rank p 0.0002). Frailty was specially associated with early death [OR 8.2 (95% CI 1.9-34) p=0.007]. In the multivariate analysis a higher risk of death was observed related to age [HR 1.07 (95% CI 1.02-1.12) p=0.002], number of frailty criteria [HR 1.13 (95% CI 1.1-1.3) p=0.05] and ISS [HR 2.6 (95% CI 1.8-3.8) p=0.001]. The frailty criteria independently associated with death were incontinence polypharmacy and previous hospital admissions. Frailty was specially associated with early death [OR 6.2 (95% CI 1.9-34) p=0.0007].

Summary/Conclusions: This study shows that the prevalence of frailty syndrome is high and has a profound impact in early death. It is also independently associated with a worse prognosis. Frailty should be considered as part of the clinical assessment when treating elderly patients with myeloma.

References:

1. E1264

Table 1.

Table 2: Univariate and Multivariate Cox Regression Analysis

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Summary/Conclusions: In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.
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 (RCT02187371) 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 further 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 (pvcSUVmean) were obtained for each lesion. The 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 analyzes 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 pvcSUVmean 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 patients with MM. The increase in pvcSUVmean 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 tumour survival, drug resistance and the development of bone disease. The Notch oncopathic pathway provides a key contribute to the ability of MM cells to shape the BM niche, affecting both BM cell biology and the intra-tumoral extracellular vesicles (EVs) that are secreted by the cells. In this work we aimed 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 contribute of EVs to the crosstalk between MM cells and the BM stromal cells.
Methods: We established two MM cell lines stably retaining the doxycycline-inducible pTRIPZ vector containing anti-Jagged1 and Jagged2 shRNAs and a BM mesenchymal stromal cell line (BMSCL) 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 conducted by Western blot analysis.

Results: We present evidences that EVs play a crucial role in the dysregulated interactions of MM cells with the BM microenvironment and that Notch regulates their release. Indeed, BMSCLs knocked down 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 expression of pro-tumor factors by BMSCL (i.e., SDF-1α), 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 BMSCLs and MM cells where the Notch pathway is blocked displays 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 (Ps) (Rajkumar et al 2010). Previous findings from 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 paradigm.

Aims: In this subsequent PREAMBLE analysis, treatment patterns in patients (pts) with RRMM receiving bortezomib (bort) and carfilzomib (carf) were evaluated to better understand the use of Ps 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 therapy and follow-up and retrospective analysis.

Results: The outcome for patients with Multiple Myeloma (MM) is highly

E1270
ROLE OF SERUM FREE LIGHT CHAIN VS BENICE JONES MEASURE-
MENT IN LIGHT CHAIN MULTIPLE MYELOMA (LCMM) AT DIAGNOSIS,
DURING TREATMENT AND FOLLOW-UP FOR RESPONSE EVALUATION
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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 “stringent” 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 measurement is usually performed within 2-8 months following the last treatment. This study aimed to assess sFLC and BJP as the primary markers of LCMM progression and the impact of these markers on outcome at diagnosis 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 were performed using Capillary II, serum and urine immunofixation using Hydrasys II (both from Sebia), sFLC were measured on Immage 800 neph-
ometry (Beckman Coulter) using Freelite 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 measurements at baseline (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 30% of pts previously treated with proteasome inhibitors (81%) and/or immunomodulating agents (40%) or chemotherapy (9%). 65% of sFLC or BJP were not 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). Bence Jones protein measurement was performed within 2-8 months following the last treatment. This study aimed to assess sFLC and BJP as the primary markers of LCMM progression and the impact of these markers on outcome at diagnosis and follow-up.

Summary/Conclusions: Both sFLC and BJP are useful in LCMMS pt for disease monitoring, however, sFLC measurement appears to be more sensitive in MDD and early relapse identification. These data suggest that plasmacytomas could be substituted by sFLC measurement in LCMMS. In our series only 1 case showed plasmacytoma occurring earlier than sFLC- PD but was considered not clinically significant. On the contrary 5 pts in both sFLC and BJP measurement was performed within 2-8 months following the last treatment. This study aimed to assess sFLC and BJP as the primary markers of LCMM progression and the impact of these markers on outcome at diagnosis and follow-up.

E1271
SUPPRESSION OF THE NON-MONOCLOAL PAIR AS NEW BIOMARKER FOR THE DIAGNOSIS OF MULTIPLE MYELOMA PATIENTS AT DIAGNOSIS
AND AFTER AUTOLOGOUS STEM CELL TRANSPLANT
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Background: The outcome for patients with Multiple Myeloma (MM) is highly
variable. Understanding the prognosis for a particular patient can help when selecting the intensity of treatment to be used and the frequency of reviews. The quantification of heavy/light chains pairs by the immunosassay Hevylite (HLC) allows us a precise measurement of monoclonal and non-monoclonal immunoglobulins of the same isotype.

Aims: The aim of the study is to evaluate i) the impact of the “HLC ratio” defined as monoclonal immunoglobulin over isotype matched non-monoclonal immunoglobulin (involved/uninvolved HLC ratio or i/u HLC ratio), ii) the suppression on non-monoclonal pair denominated “HLC-matched pair suppression” and iii) the effect of “systemic immunoparesis” at diagnosis and at +100 days after autologous stem cell transplant (ASCT).

Methods: 85 patients (50 Male:35 Female) with a median age of 70 years (56-78) were followed (35 IgGK, 18 IgGL, 17 IgAK and 15 IgAL). The median follow-up of the patients was 19 (5-30) months. Sixteen patients (18%) presented ISS stage I, 15 (28%) with stage II and 54 (64%) with stage III disease. Thirty patients that reached ASCT were evaluated at +100 days after ASCT. Immunoglobulin heavy/light chain pairs (HLC) were assessed by Hevylite assays (The Binding Site). Clinical variables were evaluated for their impact on patient’s outcome. Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan-Meier method and Cox regression. Statistical analysis was performed using GraphPad Prism 7.

Results: The median OS of the 85 patients was 54% and 26 patients deceased during the study due to MM. The median value of i/u HLC ratio was 80 (31.5-319.71). At diagnosis, an i/u HLC ratio>80 was significantly associated with worse OS (46 vs 61%, p=0.005) and shorter PFS (23% vs 42%, p=0.006). Severe HLC-matched pair suppression (i.e. more than 50% below the lower reference range) was identified in 68% of the newly diagnosed patients and was associated with significantly shorter OS (35% vs 81%, p=0.004) and PFS (21% vs 50%, p=0.013). Severe (>50%) systemic immunoparesis of non-monoclonal immunoglobulins was identified in 64% of the patients at diagnosis and was also associated with shorter OS (32% vs 81%, p=0.030) but not with shorter PFS (26% vs 44%, p=0.306). The evaluation of other clinical variables on patient’s outcome are shown in table (see Table). In multivariate analysis, severe HLC-matched pair suppression and albumin were found as independent risk factors for OS whereas creatinine and i/u HLC ratio>80 were found as independent risk factors for PFS. In the post-ASCT evaluation of the patients (n=30), normalization of HLC ratio was observed in 22 patients (73%). An altered HLC ratio was significantly associated with shorter PFS after ASCT (25% vs 70%, HR: 3.42, 95%CI: 1.12-11.97, p=0.039) and with a trend towards a worse OS (p=0.072). Severe HLC-matched pair suppression was found in 12 patients (40%) and was predictive of worse OS (0% vs 72%, HR: 10.63, 95%CI: 1.11-114.11, p=0.023) and shorter PFS (35% vs 71%, HR: 8.87, 95%CI: 1.72-45.92, p=0.002). On the other hand, the severe systemic immunoparesis observed in 17 patients (57%) was not associated with OS (p=0.644) and PFS (p=0.750).

Summary/Conclusions: Severe HLC-matched pair suppression and i/u HLC>80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. Severe HLC-matched pair suppression is an independent risk factor for OS whereas i/u HLC>80 is independently associated with shorter PFS. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

E1272

SURVIVAL STRATIFICATION OF PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER FIRST RELAPSE: SENSITIVITY ANALYSES OF A NOVEL RISK STRATIFICATION ALGORITHM (RSA)

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Background: Established risk stratification tools in MM, such as the Interna- tional Staging System (ISS) and the revised ISS, have improved overall survival (OS) estimates by combining the strongest known predictors of survival at diag- nosis. There remains, however, a need for tools that use additional data avail- able at relapse to improve risk stratification. We previously used real-world data from the Czech Registry of Monoclonal Gammapathies (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 used, as they are not routinely used; 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 started 2L. The Cox model was re-run for two sensitivity analyses: excluding CAs and adjusting for treatment received at 2L (adding bortezomib or lenalido- mide vs other treatments as a predictor). The impact of different numbers of risk groups was assessed using KAPS.

Table 1.
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 Uruguay registry designed to document clinical characteristics of newly diagnosed multiple myeloma (MM), treatment and outcomes in a real-world setting. It collects data from all institutions from all MM patients in Uruguay.

Aims: To document current strategies of clinical characteristics at diagnosis, management, outcomes and treatment adverse effects of non-selected newly diagnosed MM patients in a recent period.

Methods: This registry includes all MM diagnosed from January 2012 in all institutions, nationwide. Smoldering MM are not included. We present the analysis of the first 3 years of data collection. Information was obtained from medical records. The database includes clinical and laboratory characteristics, treatment, disease-related and treatment-related adverse events, response, progression free survival, overall survival and cause of death. Survival is obtained from the Uruguayan Ministry of Health database.

Results: With a 71% institutional coverage, 224 patients were included. Median age at diagnosis was 66 years (range 33-94 years), 54.5% were male; 10% were younger than 50 years and 34.5% older than 70 years. Distribution according Ig subtype was: IgG 50.4%, IgA 23.3%, Light chains 18.7%, non-secretory 2.2% and IgM <1%. Most patients had advanced disease: 79.6% Durie-Salmon stage III (176/221), 48.6% ISS (83/167). Anemia (hemoglobin <10g/dL) was present in 69% (31/45), osteolytic lesions in 86%, 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 7% 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% (47% in >70 y vs 41%). Most common adverse events were recurrent infections (28%), neuropathy (17%), thromboembolic events (5.4%) and grade 3-4 neutropenia (5%). Sixty out of 146 potential candidates have been transplanted as first line consolidation at the time of this analysis. After a median follow-up of 30 months, overall survival was 62.8% (median NR in ≤70 years and 32 months in >70 years) and median progression free survival (PFS) was 17 months.

Summary/Conclusions: This first national registry provides a thorough insight into the characteristics of MM patients in our country. With a high institutional coverage, we show MM characteristics at diagnosis are similar to other real-life reports.(1) MM is detected in advanced stage with a high percentage of renal impairment. Diagnosis is performed according to international recommendations. First-line treatment is defined by local policies which restrict Bortezomib to high-risk cytogenetic features and/or renal impairment and do not provide Lenalidomide. Reasons for 59% potential candidates not receiving ASCT should be addressed in future research. This analysis provides relevant real-life information to plan strategies to improve MM management and perform high quality population-based research on the field.


REPRESENTATION OF MINORITIES, THE ELDERLY AND WOMEN IN MULTIPLE MYELOMA CLINICAL TRIALS

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Background: Multiple myeloma (MM) accounts for approximately 1% of all cancers and 10% of hematologic malignancies in the United States (US). MM occurs in all races but the incidence in African Americans is two to three times higher than in non-Hispanic whites. Many clinical trials (CT) lack appropriate representation of specific 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 clinicaltrials.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% by NC1 and 25 (32%) were investigator initiated. Participation in CT varied significantly across ethnic groups, non-Hispanic Whites (NHW) were more likely to be enrolled in CT (EF of 0.23) than African Americans (AA) (EF of 0.08, p <0.0001) and Hispanics (H) (EF of 0.05, p <0.0001). Males had significantly higher enrollment than females (EF of 0.46 as compared to 0.29) with the difference being most pronounced in the elderly (EF of 0.40 as compared to 0.24) and females (EF of 0.25 as compared to 0.07).

Figure 1.
Background: Rearrangements 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, the immunoglobulin heavy chain on chromosome 14 have been associated with a worse outcome, but the clinical significance of these rearrangements is not yet clear. We evaluated a series of patients with newly diagnosed MM to determine the impact of t(11;14) on clinical outcomes and HRU in a largely community setting.

Methods: This analysis used the Connect MM registry to analyze the impact of maintenance or no maintenance of lenalidomide (LEN)-only or no LEN in the treatment of newly diagnosed multiple myeloma (NDMM) patients. The study included patients with newly diagnosed multiple myeloma (NDMM) who received lenalidomide maintenance (M) or no maintenance (N). The primary outcome was OS from diagnosis and post autologous stem cell transplant (ASCT) was 70% (p<0.0001).

Results: A total of 1493 patients with NDMM were enrolled in Cohort 1 from 2007-2011. Median age at diagnosis was 62 years, 49% pts (52%) were male, and 24% (67%) had ISS stage 3. A total of 1455 pts were evaluable for OS, and 1127 pts had OS data available. Overall, 180 (70%) pts achieved very good response (VGPR), and 243 (30%) pts achieved complete response (CR). The median OS was 30.5 months for M, and 15.6 months for N (p<0.0001). In multivariate analysis, ISS stage was an independent risk factor for mortality; pts with stage 3 had 7.3 times (CI: 1.16-36.4) and 5.7 times (CI: 1.63-20.0) the risk of mortality than pts with stage 1 and 2. Having an ASCT reduced mortality by 67% (CI: 0.04-0.41).

Conclusions: 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.

E1275
EVALUATION OF TREATMENT INDUCED PERIPHERAL NEUROPATHY IN MULTIPLE MYELOMA AND ITS INFLUENCE ON PHYSICAL AND ROLE FUNCTIONING

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Background: Peripheral neuropathy (PN) is a major dose limiting and potentially disabling adverse event of commonly therapeutic drugs used in the management of multiple myeloma (MM), including the immunomodulatory drugs (IMIDS, Thalidomide and Lenalidomide), and the proteasome inhibitor (Bortezomib).

Aims: The aims of this study were to (1) perform a psychometric evaluation of PN and (2) examine the prevalence of this complication and its influence on physical and role functioning of MM patients.

Methods: The FACT/GOG-Neurotoxicity (Ntx) subscale for assessing treatment induced PN was evaluated. The 11-item of this questionnaire was administered to patients with MM treated with IMIDS and/or Bortezomib. The subscale was evaluated in 32 patients for internal reliability, construct validity, criterion validity, and comparison with other neuropsychological and functional assessment tests (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 alpha 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 IMIDs or Bortezomib. The PN was severe in 14 (43, 7%) patients, especially those who received Bortezomib associated with IMIDS (71, 4%). PN did not influence the achievement of a very good response of MM to therapy (P=0.0005, P=0.0001 respectively).

Summary/Conclusions: PN and (2) examine the prevalence of this complication and its influence on 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 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.

E1276
PROGNOSTIC SIGNIFICANCE OF T(11;14) EXPRESSION BY FISH IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE ERA OF NOVEL THERAPIES

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Background: Rearrangements 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 these rearrangements have been associated with a worse prognosis. Further prospective studies evaluating the risk of these rearrangements are warranted.

Methods: This study (Kaufman et al, Leukemia. 2016 30:633-9) suggests that t(11;14) may confer a worse prognosis. Further prospective studies evaluating the risk of t(11;14) are warranted.

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.

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

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Background: Maintenance therapy post autologous stem cell transplant (ASCT) has been shown to improve clinical outcomes, including time to progression, progression-free survival (PFS), and overall survival (OS) in patients with newly diagnosed multiple myeloma (NDMM) (Sonneveld, J Clin Oncol, 2012; McCarthy, N Engl J Med, 2012; Attal, N Engl J Med, 2012; Palumbo, N Engl J Med, 2014; Attal, ASCO, 2016). However, the effect of continued treatment on healthcare resource utilization (HRU) is mostly unknown. Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns, and outcomes in patients with NDMM in clinical practice.

Aims: This analysis used the Connect MM registry to analyze the impact of maintenance therapy post ASCT on clinical outcomes and HRU in a largely community setting. The study (Kaufman et al, Leukemia. 2016 30:633-9) suggests that t(11;14) may confer a worse prognosis. Further prospective studies evaluating the risk of these rearrangements are warranted.

Methods: Adult patients with NDMM were eligible for enrollment in the registry within 60 days of diagnosis. Patients who completed induction and single ASCT were men, and 86% were white. Baseline patient characteristics except serum
creatinine, calculated International Staging System stage, history of monoclonal gammopathy of unknown significance, presence of del(17p), and induction regimen were similar across groups. LEN-only maintenance significantly extended PFS compared to no maintenance (median 54.5 months vs 30.8 months; hazard ratio [HR]=0.98 [95% CI: 0.43, 0.79]; P=0.0005; Table). OS was also significantly improved with LEN-only vs no maintenance (HR=0.45 [95% CI: 0.29, 0.73]; P<0.001). HRU results are detailed in the Table. The rate of hospitalization/100 person-years (PY) was similar across groups (P=not significant [NS], all comparisons) at the end of years 1 and 2. The median duration of hospitalization was numerically longer for patients who received no mainte-
ance. Procedures/surgeries and concomitant medication use were similar across both groups at the end of years 1 and 2.

Table 1.

| Serum FLC (Freelite, The Binding Site, Birmingham, UK) | GEM/PETHEMA clinical trials (GEM05menos65, GEM05MAS65, GEM2010MAS65 and GEM2012 | Results: Serum FLCs were measured by the sFLC assay (SAS-3 and SAS-4, Helena Bioscience Europe) and 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, y, k and l chains (SAS-3 and SAS-4, Helena Biosciences Europe). Results: From a total of 168 patients with NDMM (68 patients with 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 prof-

Summary/Conclusions: For patients with NDMM, LEN-only maintenance sig-
ificantly 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. 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 advan-
tege in comparison to standard quantification of MP by PEP 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.

Methods: We included 169 patients with Bence Jones (BJ) MM with measur-
able urine disease who have being treated according to GEM/PETHEMA clin-
ical trials in order to evaluate the responses and its advantages in comparison to standard quantification of MP by PEP 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.

Results: From a total of 168 patients with NDMM (68 patients with 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 prof-

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 (isFLC ≥100) being useful for its follow-up, and also provide prognostic value as a predictor of progres-

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 influ-
ence 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 benefit (class 0) from a certain treatment of interest based on the gene expression profile of the patient. This algorithm relies on the idea that geneti-
cally 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 expres-
sion profile as the prototype patients will be classified as likely to benefit from the treatment of interest. Here we use TOPSPIN to predict which patients will ben-
et from the proteasome inhibitor bortezomib. We combine tumor gene expres-
sion data from the Total Therapy 2, Total Therapy 3 and HOVON-65/GMMG–

HD4 phase III clinical trials into one dataset comprising 910 patients, split into a bortezomib arm (n=407) and a non-bortezomib arm (n=503). Progression free survival is used as outcome measure. This dataset was split in a training set (n=606) and a test set (n=304). The test set is not used at any point in the training procedure and is only used for independent validation.
Results: We successfully identify gene sets that enable us to predict which patients will benefit most from bortezomib. The top 8 performing GO sets based on Hazard Ratio (HR) were combined to achieve the final classification. In the training set 28.4% of patients are classified a class 1, resulting in an HR of 0.13 (p=7.1*10^{-11}) between the two treatment arms. More importantly, in an independent test set an HR of 0.47 (p=0.03) was found, as shown in Figure 1.

Figure 1. Kaplan-Meier plot of survival for patients treated with bortezomib and in the control arm. The survival was calculated from the date of diagnosis to the date of death or last follow-up.

Summary/Conclusions: TOPSPIN is successful in predicting bortezomib 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=12] >11 years). A significant percentage of patients had multorgan involvement (54.7% [n=75] kidney; 29.9% [n=41] nerve; 14.6% [n=20] liver; 43.8% [n=60] GI; 14.6% [n=20] skin; 22.2% [n=49] other site). Before diagnosis, 43.8% (n=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 treatment for their misdiagnosed condition. Both patients and caregivers reported correct diagnoses being made most frequently by cardioligists and hematologists (Table 1). Caregivers echoed the multitude of distinct physicians visited before diagnosis (Table 1). Patients reported that biopsy of fat pad, kidney, or heart was the predominant diagnostic test performed (Table 1). Hospitalization was prevalent; 55.5% (n=76) patients reported amyloid-related cardiac hospitalization. Moreover, 31.3% (n=43) of patients reported the need for air travel for physician consultation.

Table 1. Patient and Caregiver Survey Responses, % (n).

<table>
<thead>
<tr>
<th>Question</th>
<th>Patient</th>
<th>Caregiver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat pad biopsy</td>
<td>83.3% (n=50)</td>
<td>86.3% (n=46)</td>
</tr>
<tr>
<td>Kidney biopsy</td>
<td>81.8% (n=50)</td>
<td>83.3% (n=46)</td>
</tr>
<tr>
<td>Heart biopsy</td>
<td>80.6% (n=50)</td>
<td>83.3% (n=46)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This represents the first survey compiling both caregiver and patient experiences with AL amyloidosis. Alignment of caregiver with patient responses validates our patient-directed research. Patients with AL cardiac amyloidosis frequently receive misdiagnoses and sometimes receive incorrect treatment for the misdiagnosed condition. Disease awareness among all specialists is vital, especially among those to whom patients are initially referred due to the nature of their initial symptoms.
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 prolonged PFS and OS among patients with RRMM (see Table 1).

**Summary/Conclusions:** In the absence of prospective head-to-head trials, NMA provides potentially important information on comparative effectiveness of different treatments. This NMA suggests that the combinations of DRd and DVD are more effective than PFS in patients with RRMM with similar trends found for OS when compared with other established and new regimens.

## E1282

**TRENDS IN TREATMENT PATTERNS AND SEQUENCING IN PATIENTS WITH MULTIPLE MYELOMA DIAGNOSED 2011-2016 IN THE UNITED STATES USING AN ENHANCED ELECTRONIC HEALTH RECORDS DATABASE**

### Background:
Over the past few years, the multiple myeloma (MM) treatment (Tx) landscape has changed considerably. Immunomodulating (IMiD) drugs and proteasome inhibitors (PI) have emerged as mainstays of MM Tx. However, the limitations and lag time of available administrative claims databases make it difficult to assess current real-world trends in the Tx of MM.

### Aims:
The study aimed to describe trends in demographics, Tx patterns, and sequencing for newly diagnosed MM (ndMM) patients (Pt) in the United States (US) using an enhanced Electronic Health Records (EHR) database.

### Methods:
A retrospective observational study of ndMMPts was conducted utilizing EHR from a nationally-representative database (Flatiron Health). The Flatiron MM provider network comprises over 260 clinics throughout the US. Pts with an ICD-9 (203.0x) or ICD-10 (C90.xx) diagnosis of MM between 01/01/2011–12/31/2016 were randomly selected into the study. Pts were excluded if they did not have ≥2 documented clinical visits during the study period. Diagnosis of MM was confirmed through review of unstructured chart data. Index date was defined as the Pt’s date of diagnosis with MM. ndMM Pts were defined as those without a MM Tx more than 14 days prior to their first diagnosis date. Start of first-line (1L) therapy was defined as the 1st episode of an eligible systemic Tx given after or up to 14 days before the index date. Regimens were defined using the 1st eligible drug episode plus other eligible drugs given within 28 days of each other. A maximum gap of 90 days was allowed within a given line of therapy (LOT) and was considered concluded the day before the start date of the next LOT.

### Results:
For the 3367 ndMM Pts identified, mean(SD) age was 68.5(11) years at the time of diagnosis, 45.9% were female, 57.6% were white, 14.7% African American, and 11.1% other race. The most common immunoglobulin (ig) classes at diagnosis were IgG (51.8%) and IgA (18.9%). Median follow-up time for ndMM Pts was 15.9 months. During the study period, 1611 received only 1 line (L), 1037 were treated with 2 L, 325 with 3 L, 252 with 4 L; while 442 (13%) remained on treatment with other IMiD-containing regimens at diagnosis. Start of first-line (1L) therapy was defined as the 1st episode of an eligible systemic Tx given after or up to 14 days before the index date. Regimens were defined using the 1st eligible drug episode plus other eligible drugs given within 28 days of each other. A maximum gap of 90 days was allowed within a given line of therapy (LOT) and was considered concluded the day before the start date of the next LOT.

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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 (88.7% 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 THE ASSESSMENT IN PATIENTS WITH MULTIPLE MYELOMA WITH VGPR OR BETTER

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Background: Urine and serum Immunofixation electrophoresis (uIFE and sIFE, respectively) and free light chain assay (FLC) are widely accepted as standard tests for diagnosis and monitoring of multiple myeloma (MM). However, there is significant discordance between the electrophoretic method and FLC test for response assessment. Despite this discordance, previous studies did not address the differences in assessment of response treatment between the intact immunoglobulin MM (IIMM) and light chain only MM (LCMM/oligosecre- tory MM (OSMM)). uIFE results are poorly correlated with the serum FLC level, however, treatment response of LCMM has still been recommend to assess by 24-hour uIFE by International Myeloma Working Group guideline. However, MRD levels on uIFE negativity or normal FLC ratio (rFLC) in patients with various types of MM have not been studied.

Aims: To explore the relationship between uIFE, sIFE negativity and normal rFLC for MRD assessment in patients with IIMM and LCMM.

Methods: We initially selected 162 patients with MM (LCMM and OSMM, n = 41; IIMM, n=21) that received treatment at Kameda Medical Center, Kamogawa-shi, Japan and Kanazawa University Hospital, Kanazawa-shi, Japan between April 2008 and January 2016. Among them, 126 patients (LCMM/OSMM 40, IIMM 86), who achieve VGPR or better response, were selected on the basis of the availability of simultaneous serum and urine test, FLC data, and bone marrow MRD. To explore the relationship between uIFE and sIFE negativity and normal rFLC, MRD levels were compared by multi-colour flow-cytometry (MFC) in patients with LCMM/OSMM, and IIMM that obtained VGPR or better. MRD negativity was defined as MRD <10^-4. Complete response (CR) was divided into conventional CR (cCR, CR but MRD-positive) and MRD- CR (CR and MRD-negative).

Results: One hundred and sixty-four complete IFE, FLC, and MFC data set of 126 patients (LCMM/OSMM 40, IIMM 86) with >2 VGPR were analysed. Normal FLC at VGPR, cCR and MRD- CR was 65.0%, 78.4% and 78.6% in IIMM, and 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%/45.8% and 100%/85.0% 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 IIMM/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 AUTOALLIATEHIC STEM CELL TRANSPLANTATION

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Background: Daratumumab is a human monoclonal antibody that targets CD38, a cell surface protein that is overexpressed on multiple myeloma cells. The drug became the first monoclonal antibody as single agent therapy approved by the FDA for the treatment of multiple myeloma. The role of allo-SCT in myeloma patients (pts) remains unclear; nevertheless, the registry study of EBMT suggests an increasing rate of allografts in Europe in last years. Despite the potentially curative potential of this approach, the increased relapse rate and low PFS remain a central clinical problem.

Aims: In this single center retrospective analysis, we report on our experience on the use of daratumumab in relapsed/refractory myeloma pts after allo-SCT.

Methods: A total of 16 pts (male, n=9) with median age of 66 years (39-72) relapsing after allo-SCTs that had been performed during a period 2008-2015 at the University of Hamburg and received daratumumab as single agent sal- vage therapy. Before allografting 9 pts received one and 7 pts 2 autologous, respectively. All but one pt received at least 1 salvage therapy line prior to the allo-SCT. The allografts were performed from unrelated donors (MUD, n=9; MMUD, n=4) or matched related donors (MRD, n=3). The median of salvage lines post-transplant and prior to first daratumumab infusion was 3 (1- 4). The salvage regimens included bortezomib, lenalidomide, pomalidomide and carfilzomib. 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), muscle/skeletal pain (CTC1, n=4), acute coronary syndrome (CTC3, n=1), skin rash (CTC2, n=1), pressure on eyes (n=1). Two patients developed late onset infections (pneumonia and infection of urinary tract) followed by temporarily therapy interruption. We observed a decrease of Treg (CD4+CD25(high)Foxp3+flow) 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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Mediha, Spain, June 22 – 25, 2017

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Background: Vascular endothelial growth factor (VEGF) is a potent angiogenic peptide with biologic effects that include regulation of extracellular matrix remodeling and inflammatory cytokine generation with an important role in the bone marrow microenvironment of multiple myeloma (MM). Angiogenesis is heightened in the bone marrow of MM patients in parallel with tumor progression. Myeloma and stromal cells secrete angiogenic factors that include VEGF. Previous studies showed an increase in the expression of VEGF between plasma cells (PCs) from the same MM patient. However, no clear association with expression levels, phenotypic subtypes of PCs and prognosis was demonstrated.

Aims: The present study aimed to evaluate the expression levels of VEGF and VEGF receptor (VEGFR) on phenotypic subtypes of PCs in patients with monoclonal gammopathies and to explore its role as diagnostic and prognostic biomarkers.

Methods: We include 128 patients with monoclonal gammopathies, 60 patients with newly diagnosed symptomatic MM and 68 with monoclonal gammopathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (Chronic anemia). The expression levels of VEGF and VEGFR by flow cytometry in the two populations of bone marrow PCs, identified by gating CD138+/CD19- (clonal PCs) and CD138+/CD19+ (non-clonal PCs). The results are presented as percentage of PCs expressing VEGF/VEGFR and as expression levels of this antiangiogenic molecules expressed in mean intensity of fluorescence (MIF). The effects of these parameters on progression-free survival (PFS) and overall survival (OS) were analyzed with Kaplan-Meier method. For statistical analysis, software IBM SPSS Statistics v22 was used. ROC curves were performed to assess the VEGF and VEGF receptor accuracy as diagnostic and prognostic biomarkers.

Results: In our cohort of patients, median age was 70 (39-86) years, 52% were male. We found increased expression levels of VEGF in CD138+/CD19- PCs from MM (80±7.5 MIF) compared tomgUS patients (61±7.6± MIF) (p=0.011), and also higher to the observed in CD138+/CD19+ PCs (39.92±1.74 MIF) in both populations of patients (p<0.01 and p=0.02, respectively). No differences were observed in the expression of VEGF in CD138+ in MM compared tomgUS patients from MM (39.92±1.74 MIF),mgUS patients (41.18±1.92 MIF) and controls (32.8±1.5 MIF). However, the percentage of CD138+/CD19+ cells expressing VEGF was significantly higher in mgUS (39.4±4.4%) and in MM patients (48.7±5.5%) compared to Ch (13.5±5.5%) (p=0.019 and p=0.003, respectively). The differential expression of VEGF showed that MM patients with VEGF levels higher than constant patients (23.5 MIF in CD138+/CD19- PCs have higher probability to progress to MM [AUC 0.688 (95%CI 0.592-0.784), p<0.001, 90% sensitivity, 56% specificity, 65% PPV, 84% NPV]. In MM patients, we also found an association between increased VEGF expression levels in CD138+/CD19- PCs (11%) and survival (in the maintenance setting for example) when some inherent immune 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 (RELEN) demonstrating stable disease (SD) or better after salvage treatment with pomalidomide (POM) and low dose dexamethasone (LoDEX) induction.

Methods: Multicentre, open-label, randomized phase 2 study of relapsed RLEN patients who had received 2 prior lines of therapy. POM 4mg days 1-21 (28 day cycle) was administered alone or in combination with LoDEX (40mg weekly) as maintenance following an induction with 4 cycles of POM and LoDEX. Treatment continued until toxicity or progression. Peripheral blood samples for immune studies were collected pre-induction and prior to cycles 1, 3, 6 and 10 of maintenance.

Results: 154 patients from 15 centers were enrolled on to the study (M:F 80:74), with a median age of 67 years (range 35-85). Median number of prior lines of therapy was 4.5 (2-14). All patients had failed LLEN (100%), 127 (82.5%) were also refractory to bortezomib (double refractory) and 94 (61%) had received a prior autologous stem cell transplant. 72 (47%) patients achieved SD or better with LoDEX induction. The ORR for LoDEX induction was 35 to POM (Arm 1) and to 37 to POM-LoDEX (Arm 2). After a median follow-up of 19 months, median PFS for all patients from study entry was 4.2m (IQR 2.1 – 8.6m). PFS for randomised patients (from time of randomisation) was 2.7m for POM (arm 1) versus 3.5m for POM-LoDEX (arm 2) (p=0.39). The PFS hazard rate for Arm 2 was relatively constant compared to Arm 1 which started with a hazard rate double that of Arm 2 but dropped to less than half of the rate in Arm 2 by 15 months, suggesting that with longer follow-up, there may be an emergent advantage to maintenance with POM versus POM-LoDEX (Figure 1.). Median OS for Arm 2 was relatively constant compared to Arm 1 which started with median OS of 12.7m for POM-LoDEX (arm 2) (p=0.39). The PFS hazard rate for Arm 2 was relatively constant compared to Arm 1 which started with a hazard rate double that of Arm 2 but dropped to less than half of the rate in Arm 1 by 12 months, suggesting that with longer follow-up, there may be an emergent advantage to maintenance with POM versus POM-LoDEX (Figure 1.). Median OS for Arm 2 was relatively constant compared to Arm 1 which started with median OS of 12.7m for POM-LoDEX (arm 2) (p=0.39). 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E1290
POMALIDOMIDE 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 bortezomb 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 19% 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 haematologists 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 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% of patients had complications of these. 22.8% had hypertension and 12.5% had diabetes. Overall, 33.7% of patients were considered ineligible for transplant. Variances in patient characteristics, both by country and by line of therapy, were observed.

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% (613.3%) patients achieved CR, 13 (28.9%) VGPR and 19 (42.2%) PR. Adverse events of grade 3 or 4 included mainly anemia (4 patients), 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%, 88.6% and 100%, respectively. Forty (89%) patients had adequate stem cell collection post-ASCT (mean±SD: 8.9±4.6±10^6/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/Ang-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, as expected. RAD reduced bone resorption and increased bone formation; the latter has not been previously described with lenalidomide-based regimens. Furthermore, RAD reduced angiogenic cytokines and this supports the action of the regimen also through the disruption of the interactions between myeloma and stromal cells.

E1293
MULTIPLE MYELOMA IN THE REAL WORLD: HOW THERAPEUTIC LANDSCAPE HAS CHANGED IN THE LAST 15 YEARS
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Background: Therapeutic Multiple Myeloma (MM) scenario has completely changed in the last 30 years: conventional chemotherapy (CT) has been gradually abandoned and autologous stem cell transplantation (ASCT) is currently the gold standard treatment of multiple myeloma. Despite the clinical effectiveness of immunomodulatory drugs (IMiDs), their use in clinical practice is not homogenous and many patients do not receive a new drug and 90% after 2007 (p=0.002). Median PFS in pts >65 vs ≤65 yrs was 1.7 vs 2.4 yrs (p=0.001); median PFS in pts ≤65 yrs receiving or not ASCT was 3.2 vs 1.9 yrs (p=0.001); of note, PFS was not different when considering pts undergoing to ASCT after a CT-based or a Bor-based induction (3 vs 2.5 yrs, p=0.2). Time to next treatment (TTNT) in pts receiving ASCT or not was 30.1 months (5-122.7) vs 10.3 months (0.7-70.5) (p=0.001) from 1st to 2nd line tx and 11.2 months (0.3-121.9) vs 6.3 months (1.4-11.6) from 2nd to 3rd line tx (p=0.028). The early mortality (within the first year) was 5.9% (31/525), in details only 1/258 of those eligible to ASCT (0.4%) and 30/267 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% gastrointestinale and 18.2% hematologic toxicity; with Len-based tx 36.4% infective events and 28.9% hematologic toxicity. Median OS in pts ≤65 vs >65 yrs was 7 vs 4.8 yrs (p=0.001), of note considering pts ≤65 vs >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 has changed during the last 15 yrs. The tremendous improvement observed in this study was mainly evident in older pts with a strong reduction of early mortality and median OS reaching, in the second time frame after year 2007, 7.5 yrs. For younger pts ASCT confirmed to be of great benefit in term of TTNT and PFS. Thus, considering the real advantage of new drugs a palliative approach is not anymore justified even in very old pts.

E1294
CUL4A EXPRESSION AS A POTENTIAL PROGNOSTIC MARKER IN MULTIPLE MYELOMA PATIENTS TREATED WITH IMMUNOMODULATORY DRUGS
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Background: Despite the clinical effectiveness of immunomodulatory drugs (IMiDs) in multiple myeloma (MM), neither their mechanisms of action nor the biomarkers that could identify patients who would benefit from IMiD treatment are yet known. While the identification of the IMiD action via cereblon (CRBN), IKaros (IKZF1) and Aiolos (IKZF3) was a milestone, the role of other pathways including CRBN and E3 ubiquitin ligase complex proteins (CUL4A, DDB1, Roc1) is 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 were 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 and a positive sample was defined as ≥ 30% positive cells. Associations between studied proteins’ expression and clinical parameters were assessed using Fisher’s Exact Test for categorical variables and Mann-Whitney-Wilcoxon Test 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 progression free survival of CD138+ cells in bone marrow was observed (p=0.01) compared to low Aiolos expression, however no other associations with clinical course of MM patients were seen. No associations between IKZF1, IKZF3, IRF4, MYC expression and patients’ characteristics or outcome were revealed.
Summary/Conclusions: In conclusion, our results suggest that CUL4A expression could serve as a prognostic marker for patients assigned to IMiDs containing regimens. Further analysis of the expression of other E3 ligase complex proteins in a larger patient cohort is in progress.

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E1295
MAINTENANCE THERAPY WITH BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER ASCT AND MINIMAL RESIDUAL DISEASE (MRD)

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Background: MRD-negativity status in patients with MM after autologous stem cell transplantation (ASCT) directly correlates with higher Relapse-Free Survival. It remains unclear whereas these patients should all receive maintenance therapy with its toxicity and cost.

Aims: To assess efficacy of maintenance therapy with Bortezomib in patients with MM, who have achieved complete remission after ASCT with MRD positive and negative status.

Methods: From January 2014 to February 2016 52 patients with MM (19 male and 33 female) ages from 24 to 66 years (median 54 years) who have achieved complete remission after ASCT were randomized for a year-long maintenance therapy with Bortezomib. On 100th day after ASCT and after completion of maintenance therapy samples bone marrow from all patients were assessed using 6-color Flow Cytometry to detect MRD. We chose Relapse-Free Survival (RFS) as the indicator of maintenance therapy efficacy. Kaplan-Meier survival curves were compared using log-rank test. Statistical analysis was performed using SAS 9.4.

Figure 1.

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: 35.5–70.8%) 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=0.005).

Two year cumulative probability of relapse after ASCT in patients with MRD-negative status, who had (n=15) and hadn’t received (n=37) 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

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Background: ASCT after induction treatment has been standard of care for MM for almost 30 years. Some centers routinely perform two transplantation up-front (so-called tandem transplants), while others advocate postponing the second transplant until after progression. In recent years novel antimyeloma agents have significantly improved the prognosis of MM patients, thus casting further doubts on the value of the more aggressive tandem ASCT approach.

Aims: To describe long-term outcomes of MM patients treated with ASCT (single and tandem) in a single centre. allled 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). Seventeen percent of patients who underwent tandem ASCT are alive and progression-free more than 10 years after the procedure. Seventeen 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.
showed an association of EMD with other adverse prognosis factors and unfavorable outcomes. Our study evaluated EMD in pts undergoing autologous hematopoietic stem cell transplantation (ahSCT) are scarce.

Aims: We aimed to evaluate the clinical and laboratory characteristics of pts with EMD as well as its impact in outcomes of MM pts submitted to ahSCT (response to treatment, overall survival [OS] and progression-free survival [PFS]).

Methods: We analysed 155 MM pts submitted to ahSCT in our centre between January/2007 and December/2015, excluding second procedures. The assessment of response to treatment was based in the International Myeloma Working Group consensus criteria (2016).

Results: The median age of the cohort was 58 years (27–69), with 58% of males; the most common subtype was IgG (45%). In our cohort, 27% 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 (51% vs 40%; p<0.001). EMD was more common after 46 months (21% vs 12%; p=0.022) and without anaemia at diagnosis (28 vs 11%; p=0.023).

No other significant differences in characteristic of patients with relapse were found between 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 a 1L and 2L treatment (12.0 vs 10.8; 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 1.4; p=0.025). After a median follow-up of 48.6 months, the median an 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 1.4; p=0.025). After a median follow-up of 48.6 months, the median an 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).

Summary/Conclusions: In our cohort, EMD prevalence was significantly higher than usually described in the literature. This observation was probably associated to 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 underestimated in MM pts. It is necessary to achieve a better knowledge of the physiopathology of EMD, in order to define better treatment options that may overcome its negative impact in therapeutic response.

E1298
DIFFERENCES IN PATIENT AND DISEASE CHARACTERISTICS OBSERVED AT INITIATION OF FIRST-LINE AND INITIATION OF SECOND-LINE TREATMENT IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA IN THE CZECH REPUBLIC

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Background: Tools such as the International Staging System (ISS) and the revised ISS (R-ISS) are used to stratify risk of relapse in patients with multiple myeloma (MM), enabling assessment of survival expectations. These tools are based on factors measured at diagnosis only; understanding the role of these factors at relapse is less clear. Patient characteristics change over time; tools that rely on patient characteristics measured at diagnosis only may therefore, become less relevant than other tools that consider factors measured at relapse. The Registry of Monoclonal Gammopathies (RMG) is a large hematological disease registry, collecting data from patients in the Czech Republic and Slovakia. Data from the RMG can be used to explore real-world characteristics of the patients at diagnosis and during the course of disease.

Aims: To explore how key characteristics of patients with relapsed MM evolve between initiation of 1L treatment and initiation of 2L treatment to better understand drivers of disease progression and death.

Methods: This non-interventional, observational, retrospective study used data collected prospectively from Czech patient charts available in the RMG. Adults (218 years old) initiating 1L treatment for MM between May 2007 and April 2016 were included (N=3027); those with smoldering MM were excluded. Patient and disease characteristics were extracted at initiation of 1L and of 2L treatment. Repeated measurements were available only for those who initiated 1L and 2L treatment (1L+2L group; N=1418); patients who did not start 2L treatment may have been in remission, lost to follow-up or had died. Pts who received 1L+2L treatment, their health status improved between initiation of 1L and of 2L treatment. At 2L, patients tended to have a lower ISS stage (re-measured at 2L) than when they started 1L (stage I at 1L: 26.6%; at 2L: 41.1%). Similarly, the proportion of patients with R-ISS stage III disease was lower at start of 2L (24.6%) than at start of 1L (31.1%) treatment. Eastern Cooperative Oncology Group performance status scores were also better for patients when they started 2L than when they started 1L treatment (stage 3–4 at 1L: 8.7%; at 2L: 5.5%). Laboratory measurements indicated that patients were in better health at the start of 2L treatment than at initiation of 1L treatment: median M protein levels decreased from 31.2 g/L at 1L to 17.7 g/L at 2L, and elevated calcium and creatinine levels were less common at 2L than at 1L. Median lactate dehydrogenase levels were slightly elevated at start of 2L vs start of 1L treatment (184.4 U/L vs 206.6 U/L).

Table 1.

Summary/Conclusions: Patient health was better at initiation of 2L treatment than at initiation of 1L treatment. At relapse, patients are likely to be closely monitored and are able to initiate the next treatment line while in relatively good health; at initiation of 1L, patients may have experienced deterioration in health which could have triggered their diagnosis. These findings illustrate how patient characteristics change over time and that the underlying treatment survival may evolve; therefore, restaging patients at relapse may be beneficial and could contribute to improved predictive tools that can better define survival estimations at first relapse by considering patients' experiences at 1L.

E1299
AN EARLY GOOD RESPONSE AFTER BORTEZOMIB-BASED INDUCTION REGIMENS REPRESENTS A SIGNIFICANT PREDICTOR FOR IMPROVED PFS IN NDMM PATIENTS

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Background: Introduction of triplets-based induction regimens containing proteasome inhibitors (PIs) in clinical practice have led to higher response rates and prolonged life expectancy in newly diagnosed multiple myeloma (NDMM)
patients. Different studies have linked complete response (CR) with better PFS (progression free survival), but not always with prolonged overall survival (OS), most likely due to the impact of novel agents in the management of relapsed-refractory patients. Overall, these observations suggest PFS as a more reliable predictor of clinical outcome. Also, the biological aggressiveness is emerging as a pivotal disease characteristic which affects clinical behavior and response to therapy. In this context, little is known about the association of response kinetic with survival outcomes.

Aims: In order to evaluate whether early achievement of a good quality response impacts on outcome, we retrospectively analyzed 87 NDMM patients treated at our institution with bortezomib containing regimens (BRs).

Methods: From 2004 to 2016, 87 patients with NDMM and measurable disease (serum and/or urine M protein) were treated with BRs. Both patients eligible and non-eligible for ASCT were included in the study; patients undergoing ASCT were censored at the time of transplant. Median age was 66 (range 32-87); males were 51 (59%); 72 (83%) patients were in 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).

Methods: Individual patient-level data (IPD) were reconstructed from the published Kaplan-Meier PFS curves from the ELOQUENT-2 (ERd), ASPIRE (KRd), and TOURMALINE-MM1 (NRd) randomized, controlled, Phase III trials using digitization software and the methods described by Guyot, et. al. Using the reconstructed IPD, Kaplan-Meier survival curves were estimated for each arm within each trial. PFS curves were digitized by two independent researchers and the reconstructed curves were overlaid with the published data to validate the IPD. In each trial, the relative PFS benefit over time was calculated as the difference in the Kaplan-Meier PFS estimate of each triplet regimen and the Kaplan-Meier PFS estimate of Rd divided by the Kaplan-Meier PFS estimate of Rd: rPFS(t)= (S_E(t) - S_R(t)) / S_R(t). Where S(t) denotes the Kaplan-Meier PFS estimate at time t, and L 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

POMALIDOME 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 (RRMM) 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 retrospective analysis in a population-based registry was conducted to assess response and survival in patients with RRMM 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/or an immunomodulatory 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. The 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).

Summary/Conclusions: For the treatment for RRMM, ERd showed an early and sustained benefit in relative PFS which was maintained through 40 months. KRd and NRd showed initial benefits which faded by the end of data availability.
Results: A total of 82 patients (median age 68 years [range: 43-88]) were included in this analysis. CRAB criteria included anemia in 23 patients (28%), renal insufficiency in 8 (9.8%), hypercalcemia in 13 (16%) and bone lesions in 54 (66%). Median time from diagnosis to start pomalidomide was 5.75 years [range: 0.8-18.4]. median number of treatment cycles was 3 [range: 1-17]. At time of analysis 59 patients had stopped pomalidomide treatment: 24 patients had progressive disease, 10 had unacceptable toxicity, 6 patients were refractory, 4 patients died during treatment and 15 patients stopped due to various other reasons. Grade ≥3 hematological adverse events occurred in 11% of patients, 4% had neutropenic fever. Grade ≥3 non-hematological toxicities occurred in 57% of patients, including infection in 22%, gastrointestinal disorders in 5% and renal disorders in 5%. Of 69 patients evaluable for response ORR was 41%, with a partial response (PR) rate and a very good partial response (VGPR) rate of 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 2.3-8.0) 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 1.3-6.8) 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: Our results show that older patients and patients with a long interval between initial diagnosis and pomalidomide treatment (indicating a less aggressive 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 APBSCT. 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) par quotient quantification, sFLC and total immunoglobulins levels in serial serum samples collected during the follow-up. Involved/uninvolved index (I/Ui) was calculated using the monoclonal chain (Involved) as numerator and the polyclonal chain of the same class (Uninvolved) as denominator. The HLC ratio (HLC) was calculated as IgGx/IgGA or IgAx/IgAA 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 in the uninvolved chain. 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-par 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.

Figure 1.

Summary/Conclusions: In this analysis the experience in clinical practice of patients with RRM 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.

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 8-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 iCR, 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 iCR and 47.6% (10/21 patients) not in iCR relapsed during the follow up. Patients in iCR showed significantly longer PFS with median 42 months than those in less than iCR with PFS median 29 months (p=0.0196, log-rank test). This was reflected in a hazard ratio of relapse (0.3865) lower in iCR compared to CR. The 5 year OS was 91% in iCR and only 75% in CR. An improvement of PFS and OS was also noted in patients achieving iCR versus CR (26.3% vs 16.1% respectively).

Summary/Conclusions: Achieving immunophenotypic CR is clearly associated with longer progression free survival compared to conventional CR. Reaching iCR should be a goal of myeloma treatment.

E1304
REGULATION OF NORMAL AND MONOCLONAL IMMUNOGLOBULIN SECRETION BY CYTOKINES (S-SYNDECAN-1, BLYS & TGF-BETA-1) IN PATIENTS WITH IG-SECRETING B-CELL DISORDERS AT PRESENTATION.

PROGNOSTIC IMPLICATIONS

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Background: The most common neoplastic lymphoproliferative diseases that secrete paraprotein are multiple myeloma (MM), Waldenstrom’s Macroglobulinemia (WM) and chronic lymphocytic leukemia (CLL). The two first entities secrete paraprotein by definition, while serum free light chains (sFLC) are increased in 50% of CLL cases. Microenvironmental factors, such as soluble Syndecan-1 (ssynd-1) and BlyS normally promote lymphoplasmacytic differentiation as well as their secretory activity, whereas, like TGFBeta1, inhibit them. 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 ‘Heavylite’ method (that measures the amount of secreted Ig does not really reflect disease burden. The heavy chain Ig can be accurately determined with the ‘Heavylite’ method (that measures the amount of secreted Ig). However, the light chain isomer (kappa or lambda), thus allowing exact quantification of the amount of pure monoclonal fraction but also the degree of suppression of polyclonal lgs, both being reflected by the corresponding ratios (HLCR).

Aims: To determine any possible relationship between the amount of lgs secreted and the degree of suppression of polyclonal lgs 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-ISS 1, 2, 3 respectively), and 100 with CLL (67%, 23%, 10% staged Binet 1, 2, 3 respectively). Patients were regularly followed up at least last visit or (median follow up 5 months). sFLC/sFLC R and HLCR/HLCR R 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 lg 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 progression. By inhibiting both monoclonal and polyclonal lg, 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 (LT-CR) 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 (LT- CR). Evaluation 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-LTCR patients.

Methods: After approval by the ethics committee, we selected 13 patients diagnosed with MM, in sCR according to IWG 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. Mibian follow up. B-cells after APBSCT was 8 years (range 6-19). Group HA: 5 females and 10 males, median age 60 (36-78). Immune phenotypic characterization was done using a comprehensive 8-color flow cytometry panel. Subpopulations of CD4+ and CD8+ T-cells from PB were quantified, including naïve, central and effector memory T-cells, as well as subpopulations of B-cells: naïve, transitional, marginal zone-like, class-switched memory and plasmablasts. In order to confirm their specific immune signature, the analysis was repeated in the same LT- CR-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 same group “patients +1 year”. Statistical analysis was done using GraphPad Prism software.

Results: The patients had a lower percentage of total CD4+ T-cells (p=0.0004) together with a decrease in the naïve CD4+ T-cells (CD27-CCR7+CD45RA+; p=0.0004) and an increment of the effector memory CD4+ T-cells (CD27CCR7+CD45RA-; p=0.0028). In addition, both CD72CCR7CD45RA+ and CD72CCR7CD45RA+ similar results were found within the CD8+ T-cells. No differences were observed in the Tregs defined as CD4+CD25+ICD127. The mean percentage of total B-cells in the patients was within the normal range and no significant differences were found when compared to HA. However, naïve B-cells (CD22-CD19-CD7+) proportion was higher in patients and a corresponding reduction of marginal zone-like B-cells (CD22-CD19CD7-CD23+) and plasmablasts (CD27++CD38++)(p=0.0047) was observed. No differences were observed in the percentage of transitional B-cells (CD27-CD10-CD38+) or plasmablasts (CD27+ CD38++) in the PB of the two groups. When the analysis was repeated in the same LT- CR-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-LTCR patients seem to express a distinctive immune “footprint” characterized by a decreased proportion of naïve T-cells and an increased percentage of effector T cells, which probably exert a competent immune surveillance. Conversely, the increase in naïve B cells may guarantee the humoral response homeostasis, inhibiting the recognition of normal plasma cells that might compete with myelomatous cells for normal bone marrow niches. The precise role of these refined immunological studies in the monitoring and therapeutic decisions in MM patients, and also in the duration of sCR, should be defined in the future.

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E1306
IMPACT OF THE AFFORDABILITY OF NOVEL AGENTS IN PATIENTS WITH MULTIPLE MYELOMA: REAL WORLD DATA ON CURRENT CLINICAL PRACTICE IN MEXICO

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 agents and transplantation settings in public health services.

**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, with 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 vs. 42 months, p<0.05) and ineligible patients (median 79 vs. 46 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.

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**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⁺ fluctuates during normal megakaryopoiesis and how CALR mutations could affect the basal Ca²⁺ levels in megakaryocytes in MPNs.

**Methods:** Ca²⁺ staining was performed using Fluoro-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⁺ in MPNs affected by basal Ca²⁺ reduction which could be essential for megakaryopoiesis. In order to understand how CALR mutations affect basal Ca²⁺, Marimo cells and Dami cells expressing CALR mutations were analysed. Here we show a decrease in basal Ca²⁺ in Marimo cells and Dami-CALR type2 mutation compared to the controls. Moreover, Dami–CALR type1 did not show any significant reduction, suggesting possible differences in Ca²⁺ behaviour in MPNs. We are currently working in the analysis of basal Ca²⁺ fluctuations during megakaryopoiesis in the presence of CALR mutations and preliminary results show abnormal basal Ca²⁺ levels throughout all the process of megakaryocyte differentiation.

**Summary/Conclusions:** Altogether, our findings indicate that basal Ca²⁺ could be an important element during megakaryopoiesis and CALR mutations found in MPN could impair the normal production of megakaryocytes due to changes in cellular Ca²⁺. However, further analysis need to be done in order to understand the role CALR mutations and their effect in the Ca²⁺ buffering activity of CALR in MPNs.

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**E1308**

**THE INHIBITION OF JAK/STAT SIGNALING IS COMPENSATED BY ACTIVATION OF MAPK PATHWAY IN MYELOPROLIFERATIVE NEOPLASMS**

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**Background:** Myeloproliferative neoplasms (MPN) remain incurable regardless of advancement in the use of 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-bromocyclodexane 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 Hexabromocyclohexane 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 agonists 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 distinct abnormalities in megakaryocytic (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 spleenomegaly and constitutional symptoms in about 50% of patients (pts). We hypothesized that, 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-antiagglutinated peripheral blood from healthy donors (HD, n=10) and JAK2V617F positive MF pts (n=12) at intermediate-2/high IPSS criteria was collected at baseline and 3 and 6 months after RUX therapy and immediately centrifuged. Microparticles in megakaryocytic (MK) development and platelet (PLT) activation. Microparticles were qualitatively and quantitatively assessed by flow cytometry (CytoFLEX, Flow Cytometry- Beckman Coulter). The instrument was calibrated with MEGAMIX Beads (Beckman Coulter) with various diameters to cover the MPs (0.5 and 0.9μm).

**Results:** At 3 and 6 months, 5 out of 12 pts achieved a spleen response (R) according to 2013-IWG-MRT criteria. At baseline, the mean percentage of MK-derived MPs was significantly decreased (29±6 vs 72±5; p<0.001) while that of PLT-derived MPs significantly increased (49±7 vs 11±1; p<0.001) in MF pts compared to HD. However, the mean percentage of MK-derived MPs from pts not achieving a spleen response (NR) was significantly decreased compared to R (72±5; p<0.001) and HD (57±4 vs 72±5; p<0.001). By contrast, the mean percentage of PLT-derived MPs was significantly increased in NR compared to R (64±7 vs 37±9; p<0.05) and HD (64±7 vs 11±1; p<0.001). Of note, NR pts had significantly lower PLT number as compared to R (220±29 vs 422±98 p<0.05) and HD (72±5; p<0.0001). No correlation was observed at baseline between the percentages of MK/PLT-derived MPs and platelet number, allele burden, spleenomegaly and constitutional symptoms. At 3 and 6 months, RUX did not significantly modify the mean percentages of MK- and PLT-derived MPs compared to baseline values.

**Summary/Conclusions:** At variance with HD, the majority of circulating MPs in JAK2V617F mutated MF pts at intermediate-2/high IPSS risk derived from PLTs. RUX therapy did not modify the MK/PLT-derived MPs pattern, suggesting that JAK1/2 inhibition does not seem to affect the pathways of MK/PLT MPs production or closure. Most importantly, MPs evaluation at baseline is significant, especially when associated with subsequent spleen response. Specifically, NR pts had increased percentages of PLT-derived MPs with a concomitant reduction of PLT number. This could be related to a state of PLT hyper-activation with hyper-aggregation responses. Further studies are needed to confirm whether MPs may actually be considered a biomarker of disease activity and response to RUX.

**E1310**

**A COMPARATIVE FUNCTIONAL AND PHENOTYPIC PLATELET ANALYSIS AMONG GENETIC GROUPS OF ESSENTIAL THROMBOCYTHEMIA PATIENTS**


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**Background:** A number of genomic abnormalities have been associated with primary myelofibrosis (PMF). Non-coding region sequencing (NGS) and single-nucleotide polymorphism array (SNP-A) methods are currently used for PMF genomic studies and certain cytogenetic and genomic abnormalities 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 genotypic variations 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). SNP-A and NGS data analyses were performed using Illumina BaseSpace Informatics suite (Illumina). JAK2, CALR, MPL mutations were additionally confirmed with Sanger sequencing while small indels – with DNA fragment analysis.
Results: 110 patients diagnosed with PMF according to WHO criteria between years 2013 and 2014 were included into 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: 5q loss (55.7%), 20q deletion (11.5%), 1q duplication (4.9%), 19p deletion (2.4%), 1p deletion (3.2%) and 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, ET6, 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”. Previously not described ZRSR2 gene 12 bp insertion was indentified in four patients (3.7%). The correlation analysis showed significant associations between 5q LOH and JAK2(V617F)mutation (p=0.010); ASXL1 mutations (p=0.011); 19p deletion and CALR mutations (p=0.004). Notably, the affected genes are located in core-sponding affected chromosome regions, indicating disruption of both alleles by different biological mechanisms. KRAS and ET6 mutations were statistically associated with ASXL1 mutations (p<0.001 and p=0.005, respectively) while JAK2 and CALR mutations were mutually exclusive in all cases (p=0.001).

Summary/Conclusions: A number of associations between gene mutations and chromosomal aberrations was revealed in PMF. Co-presence of 5q LOH with JAK2(V617F) and CALR mutations with 19p deletion indicate that further deregulation of these key signaling pathways may take place disrupting the second allele of the affected genes by different biological mechanism – LOH or deletion.

E1312

FREQUENCY OF CONCURRENT BCR-ABL1, JAK2, CALR AND MPL MUTATIONS IN A COHORT OF 5,545 CASES WITH SUSPECTED MPN BY A DEEP SEQUENCING APPROACH

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neo-plasm which is characterized by a bcr-abl1 rearrangement in almost 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 9x109/L, hemoglobin level (Hb) was 15g/dl, and platelet count was 328x109/L. All of these cases were analyzed by an amplicon deep sequencing approach for mutations in JAK2 (exon12, exon14), CALR (exon9) and MPL (exon10) with a sensitivity of 1%. 3070 patients were additionally screened for BCR-ABL1 fusion by a multiplex PCR approach. Samples that were double mutated for JAK2, CALR and MPL were analyzed by amplicon deep sequencing for additional mutations in 13 myeloid genes.

Results: In total 1775/5545 (32%) of suspected MPN patients showed JAK2, CALR and/or MPL mutations. 1438 (26%) were JAK2, 267 (5%) CALR, and 89 (1%) MPL mutated. Of note, the analysis of a subgroup (n=3070) for BCR-ABL1 fusion identified 123 (4%) as CML cases. The JAK2 mutated cases presented mainly with Va817PhE (99%) and rarely with JAK2 exon12 mutations (1%). CALR mutations were primarily type 1 (54%) and type 2 (30%). MPL mutations were located at amino acid Trp515 in 96% of cases. Double mutated cases were present in 19/1775 (1%) cases: JAK2/MPL (63%), JAK2/CALR (32%) and CALR/MPL (6%). In nearly all CALR mutated cases (67%) the CALR mutation was detected with the higher load, whereas in JAK2/MPL double mutated cases the ratio was equal. Most of the patients (18/19) had one mutation with a load below 10% and could have been missed by other approaches. BCR-ABL1 together with JAK2 or CALR mutation was found in one patient, each (0.21%). In 64% of the 267 patients, out of the 267, dated the previous 2-3 years, 8 patients had already received treatment for CML but were suspected to have independent BCR-ABL1-negative MPN. For two of these patients, samples 1 and 6 years prior to diagnosis of CML were available. Both showed CALR mutations already at this former time-point at high loads. In 10/19 (53%) double mutated patients the CALR mutations were detected in 8 different genes. SRSF2 and TET2 were the most frequently mutated ones (n=3, each). No significant difference in mutation frequency was detected to the overall frequency in MPN patients with single mutations. The JAK2, CALR and/or MPL mutated vs wild-type cases showed higher age (mean: 67 vs 56 years, p<0.001) and higher platelet count (480 vs 260x109/L, p<0.001). Interestingly, out of the 267, overall, the p-value was significantly different according to the presence of mutations as follows: triple-negative (56 years), CALR (63 years), JAK2 (67 years), MPL (71 years) and double mutated (74 years).

Summary/Conclusions: One-third of the cases can be diagnosed having BCR-ABL1 fusion 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. The 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 TANSLX1mut. 3.6 years in TANSLX1wt, 13.8 years in DM(+)TANSLX1wt and was not reached in DM(+)TANSLX1mut (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, р=0.0005). There were no differences in OS of ASXL1 wt- HR, ASXL1 mut-LR and ASXL1 mut-HR pts (1.4 vs 1.6 vs 1.2 years, р=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. OS in pts with any of the findings: HR karyotype or ASXL1 mut – was significantly shorter than in cytogenetically favorable ASXL1 wt 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-Larrán A e.a. Leukemia Research 2012, 36(3):324-326]. However, the data on the impact of the haplotype on the JAK2V617F 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 [Krichesky S e.a. Blood Cells, Molecules and Diseases., doi: 10.1016/j.bcmd.2017.01.001]. Influence of haplotype 46/1 for such cases is not known. There are two competing hypotheses of "hypermutability" and "fertile ground" explaining the causes of the higher frequency of mutations of JAK2V617F in haplotype 46/1 carriers. The "hypermutability" hypothesis refers to an increased risk of a primary mutation in carriers of haplotype 46/1. In this case, the increasing frequency of the haplotype in patients with low allele burden (<5%) must also be observed, including those individuals without evidence of hematological disorders. Aims: Studying the relations of haplotype 46/1 and JAK2 V617F allele burden Methods: The diagnosis of chronic myeloproliferative neoplasms was based on the WHO (2008) criteria. The cohort included patients with JAK2 V617F mutation: 100 patients with PV, 51 with ET, 14 with MF, 41 patients with unclassifiable MPN, 51 with PMF. Patients with JAK2V617F allele burden of 100% were excluded. The JAK2V617F allele burden was evaluated by a quantitative real-time allele-specific PCR test using "in-house" kit (0.01% sensitivity). Associations between JAK2 V617F mutation and haplotypes of JAK2 were assessed using the Chi-squared test. The odds ratio (OR) and 95% confidence interval (CI) were also calculated for each comparison of JAK2 46/1 haplotype frequency and ranges of allele burden.

Results: The JAK2 46/1 haplotype (GG and CG) was present in 170 patients (80.6%) with MPN, in 25 (52%) patients with suspected MPN, in 23 (49%) asymptomatic JAK2 V617F+ patients and in 42 (42%) cases of control group. G variant of rs10974944 was more frequent in all JAK2 V617F-positive MPNs, than in the control population (χ²=46.5, p<0.0001). These results were similar to findings of previous studies, which have shown that the 46/1 haplotype predisposes to the acquisition of JAK2 V617F mutation. JAK2V617F allele burden was significantly higher in patients with PV than in patients with ET (p<0.001), but no differences were observed with from patients with the PMF. 46/1 haplotype was closely associated with MPN patients if the allele burden exceeds 5% (Fig. 1) regardless of the phenotype or the treatment. In this case with an increase in JAK2V617F allele burden the JAK2V617F allele burden significantly increased. However, there was no significant difference in the JAK2 46/1 haplotype frequencies between patients with allele burden less than 5% and the control group.

Summary/Conclusions: No significant differences of the carrier haplotype frequencies between control group and patients with minimal allele burden (less than 5%) JAK2 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 ALLOGENIC STEM CELL TRANSPLANTATION (ASCT)

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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 disease can vary from mild to many patients to a very aggressive form. 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 both by conventional allele specific PCR (ASO-PCR) and by a validated ddPCR mutation detection assay (Bio-rad, USA). Results were expressed as percentage of JAK2V617F mutated alleles on total evaluated alleles.

Results: The JAK2V617F ddPCR mutation assay was able to detect low muta- tion load (up to 0.006%), confirming to be much more sensitive than ASO-PCR (0.5-2%). In 4 patients, early after transplantation, we observed by ddPCR a low level of MRD that progressively increased during the follow-up and antici- pated a decrease in donor chimerism level and a worsening of clinical situation. In 2 patients, who showed a full donor chimerism and complete hema- tologic remission of the disease, very low levels of MRD (ranging from 1% to 0.06%) could be detected by ddPCR in the 2 years after ASCT. With a longer follow-up, a full molecular remission was achieved as demonstrated by ddPCR. In 2 other patients, we observed a very early achievement of full donor chimerism and JAK2V617F molecular negativity (within 90 days post HSCT), also when evaluated by ddPCR. These patients entered a complete hematologic remission of the disease which still persists (after 1 and 5 years after transplantation, respectively). Interestingly, in one patient whose post-transplant hematopoiesis proved full donor and negative for JAK2V617F mutation for 2 years, a weak positive signal revealed by ddPCR (0.075%) became apparent at 12 months after ASCT and anticipated an extra-hematologic relapse (skin and bone). A subsequent second allogeneic transplant from the same sibling donor restored clinical and molecular remission.

Summary/Conclusions: The ddPCR proved to be a sensitive and accurate method in detecting JAK2V617F mutation. Therefore, this assay can be a valid tool for MRD monitoring in MF and a candidate for ASCT. However, the use of this highly sensitive PCR should be considered with caution in the clinical management of transplanted patients to avoid inappropriate use of donor leukocyte infusion (DLI) and tampering of immunosuppression. A large,

Figure 1.
number of patients have to be studied with ddPCR to better understand the clinical significance of low mutation load.

**E1316**

**S100A8/9 ACTIVATION OF MAPK PATHWAY IS SUPPORTED BY ITS RECEPTORS RAGE AND TLR4 IN POLYCYTHEMIA VEREA**

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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 excessively augmented in myeloproliferative neoplasms (MPN). S100A8/9 is involved in signal transduction, cell transformation and cell growth. It has been shown that S100A8/9 factor, further on ameliorated by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated MAPK activation has been significantly augmented by TLR4 and RAGE activation of AKT and MAPK pathways by S100A8/9 proteins in healthy controls and MPNs: polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), according to JAK2V617F and calreticulin (CALR) mutation status.

**Methods:** S100A8/9 factor is examined in granulocytes of MPN using immunoblotting, while its influence on cell proliferation 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: S100A8/9/Anteiles demonstrated a common significant increase in plasma of MPN patients, whereas the presence of CALR mutation augmented S100A8/9 levels in granulocytes of ET and PMF patients. Activation of AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by inhibition of the receptor for advanced glycation end products (RAGE) in granulocytes of JAK2V617F+ and JAK2V617F-/CALR+ groups of ET and PMF patients, while it has been prevented by Toll-like receptor 4 (TLR4) inhibition in PV patients. MAPK pathway is significantly inhibited by S100A8/9 only in JAK2V617F+ ET patients and JAK2V617F-/CALR- PMF patients, partially prevented by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated AKT and MAPK activation, while in contrast, S100A8/9 mediat ed MAPK activation has been significantly augmented by TLR4 and RAGE inhibition in PV patients. S100A8/9 stimulated granulocyte cycle arrest in G2M phase has been stopped by JAK2/1 inhibition.

**Summary/Conclusions:** S100A8/9 protein levels demonstrated stable elevation in MPNs, while inhibition of AKT pathway has been controlled by TLR4, whereas MAPK pathway activation by TLR4 and RAGE in PV, during treatment with S100A8/9.

**E1317**

**MUTATIONAL PROFILE STUDY OF DOUBLE-NEGATIVE ESSENTIAL THROMBOCYTHEMIA BY HIGH-DEPTH NEXT GENERATION SEQUENCING (NGS)**

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**Background:** Essential thrombocythemia is one of the three classical phae- noma 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 of the patients.

**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 thrombotic event after diagnosis, 1 patient suffered transformation to AML. Median Hb, WBC and platelets at diagnosis were respectively 14.75±0.5; 8.5±0.1x109/L and 720±10x109/L. We performed targeted gene sequencing by NGS (Ixon Torrent Proton System–Life Technologies) using a panel of 33 genes implicated in leukemia progression. 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-synonymous mutations which were used to find association between mutations and clinical data.

**Conclusions:** 540 patients have been regulated to state cell proliferation, excessively augmented in myeloproliferative neoplasms (MPN). S100A8/9 is produced by cells of myeloid origin as mediator of inflammation, while AKT excessively augmented in myeloproliferative neoplasms (MPN). S100A8/9 is involved in signal transduction, cell transformation and cell growth. It has been shown that S100A8/9 factor, further on ameliorated by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated MAPK activation has been significantly augmented by TLR4 and RAGE activation of AKT and MAPK pathways by S100A8/9 proteins in healthy controls and MPNs: polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), according to JAK2V617F and calreticulin (CALR) mutation status.

**Methods:** S100A8/9 factor is examined in granulocytes of MPN using immunoblotting, while its influence on cell proliferation 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: S100A8/9/Anteiles demonstrated a common significant increase in plasma of MPN patients, whereas the presence of CALR mutation augmented S100A8/9 levels in granulocytes of ET and PMF patients. Activation of AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by inhibition of the receptor for advanced glycation end products (RAGE) in granulocytes of JAK2V617F+ and JAK2V617F-/CALR+ groups of ET and PMF patients, while it has been prevented by Toll-like receptor 4 (TLR4) inhibition in PV patients. MAPK pathway is significantly inhibited by S100A8/9 only in JAK2V617F+ ET patients and JAK2V617F-/CALR- PMF patients, partially prevented by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated AKT and MAPK activation, while in contrast, S100A8/9 mediated MAPK activation has been significantly augmented by TLR4 and RAGE inhibition in PV patients. S100A8/9 stimulated granulocyte cycle arrest in G2M phase has been stopped by JAK2/1 inhibition.

**Summary/Conclusions:** S100A8/9 protein levels demonstrated stable elevation in MPNs, while inhibition of AKT pathway has been controlled by TLR4, whereas MAPK pathway activation by TLR4 and RAGE in PV, during treatment with S100A8/9.
Background: We already demonstrated augmented proinflammatory IL-6 and angiogenic vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α) and endothelial nitric oxide synthase (eNOS) levels in myeloproliferative neoplasms (MPN).

Aims: To observe IL-6 activated signaling pathways during stimulation of angiogenic factors and their JAK-STAT dependence in MPN.

Methods: We analyzed phosphorylation of JAK/STAT3, PI3K/AKT and MAPK signaling by immunoblotting in HEL 92.1.7 cells (with JAK2V617F mutation) and granulocytes of MPN. The granulocyte cycle phases have been studied by flow cytometry.

Results: We demonstrated IL-6 stimulated angiogenic factors in HEL cells and HEL-derived macrophages, blocked by JAK-STAT inhibition for eNOS and HIF-1α. IL-6 stimulated JAK-STAT3 and angiogenesis related PI3-AKT signaling pathways in HEL cells, the later one prevented by JAK1/2 inhibition. Opposite to primary myelofibrosis (PMF), IL-6 activation of JAK-STAT3 and PI3-AKT pathways has been prevented and enhanced by JAK1/2 inhibition, respectively in granulocytes of polycythemia vera (PV). Moreover, IL-6 inhibition of JAK-STAT3 and PI3-AKT pathways in essential thrombocythemia (ET) has been prevented by JAK2 inhibitor in JAK2V617F positive ET granulocytes. JAK1/2 inhibitor Ruxolitinib upregulated IL-6 activators of MAPK pathway in MPN, in contrast to specific JAK2 inhibitor Hexabromocyclohexane. IL-6 mediated reduction in the percentage of HEL cells in G2M phase was reversed by Ruxolitinib that potentiated apoptosis and reduced the cell percentage in GO/G1 phase both in HEL cells and granulocytes of PMF. It has been detected the cell cycle arrest of MPN granulocytes in S phase (DNA replication) after treatment with IL6, completely diminished by JAK1/2 inhibition.

Summary/Conclusions: Therefore, we concomitantly revealed that inflammation stimulated angiogenic factors and signaling pathways involved in cell proliferation, apoptosis and angiogenesis are regulated by JAK-STAT inhibition.
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.

Methods: Data from the COMFORT-I trial of ruxolitinib (Verstovsek 2012) versus placebo was obtained from Incyte® for independent analysis. Association of total symptom burden (TSS; divided by the sample quartiles) and QOL (divided by the sample median) at baseline with OS among MF patients was estimated using the Kaplan-Meier method and tested using log rank tests and Cox regression. Symptom burden and QOL were assessed using the 5-symptom MF-SAFv2.0 (Mesa 2009) and EORTC QLQ-C30 Global Health/QOL scale (Aaronson 1993). In this population-based study, pts were not included in this symptom burden assessment and may represent an independent factor associated with increased survival.

Results: A total of 309 patients were available for analysis including 155 ruxolitinib-treated and 154 placebo-treated MF patients. Baseline demographics, disease-related variables, and calculated overall survival were similar to previous published results (Verstovsek 2015). Symptom Burden: When comparing OS by TSS quartiles at baseline, no significant associations in OS were observed (Figure 1A). Individual symptoms of bone or muscle pain, feeling full, pain under ribs on left side, abdominal discomfort, itchiness, or night sweats did not demonstrate significant associations when comparing OS by quartile symptom score. Baseline fatigue score demonstrated no difference in OS when stratified by median or quartiles. Global Health Status/QOL: To determine if treatment had an impact on QOL, the EORTC QLQ-C30 (Cella 2007) was administered. QOL components that showed significant differences between treatment groups included global health status (p < 0.01), fatigue (p < 0.001), nausea and vomiting (p < 0.02), and appetite (p < 0.03). Cox Proportional Hazards Modeling: Cox regression analysis reached significance for items of age (p < 0.001), sex (p < 0.001), 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 mp-MF high IPSS. This study suggests using QOL as a prognostic tool for symptom burden, disease risk, age, sex, and treatment. Prior literature has confirmed the importance of QOL in prognosticating survival in other cancer types. However, this is the first study that has identified the key correlation among individuals with MF. Neither individual nor combined symptom scores at baseline appeared prognostic for overall survival, emphasizing the importance of QOL assessment in addition to symptom assessment. Weight loss (a prognostic factor for DIPSS scoring) was not included in this symptom burden assessment and may represent an independent factor associated with increased survival.

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, Nils- 

Aims: To evaluate the prognostic relevance of QOL and symptom burden among patients with MF from the COMFORT-I study.

Methods: We identified a total of 3,367 pts with primary myeloid fibrosis (PMF, ICD-O-3 morphology code as 9961/3 and primary site code as C420, C421 or C424) diagnosed between January 2000 to December 2013. Pts with missing survival status (n=753), pts lost to follow up (n=4), and pts with missing age record (n=1) were excluded. Kaplan-Meier analysis was performed to determine overall survival (OS) and cancer specific mortality. The effects of specific covariates on OS were analyzed using a Cox proportional hazards model.
Results: The final study cohort comprised of 2,619 PMF pts. Median follow up period was 28 months (interquartile range (IQR) 11-56). Median age at diagnosis was 68 years (interquartile range 59-77 years) with 60.6% (n=1,586) ≥ 65 years old. More than half of the pts were male (58.5%; n=1,531); 82.2% (n=2,153) were white, and 16.4% (n=430) were diagnosed between 2012 and 2013. The geographic distribution was as follows: East 14.8%, South 18.4%, West 54.2% and Midwest 12.6%. Median OS was 42 months (Figure 1). The hazard ratio of all-cause mortality for age was 1.05 (95% Confidence interval (CI) 1.04-1.05), for female vs male was 0.72 (CI 0.64-0.80), for nonwhite vs white 1.01 (CI 0.87-1.16), for unmarried vs married was 1.04 (CI 0.94-1.16), for patients diagnosed 2012-2013 vs 2000-2011 was 0.95 (CI 0.75-1.20). Compared to West, the hazard ratio of OS for East, South and Midwest was 1.05 (CI 0.90-1.22), 1.28 (CI 1.12-1.47), 1.03 (CI 0.88-1.19) respectively.

Summary/Conclusions: This population based study showed that the overall survival of pts with PMF was short. Older and male pts were associated with higher mortality risk. There were significant differences across geographic regions of the United States. Although there is a trend of improvement in the period of 2012 to 2013, the result is not statistically significant, partially due to short follow up. These findings underscore the continuing need for effective therapies for pts with MF.

E1323

SERUM ALBUMIN IS A STRONG PREDICTOR OF SURVIVAL IN MYELOFIBROSIS, INDEPENDENT OF IPSS, DIPSS, AND DIPSS+ SCORES

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Background: Albumin is the main protein in human plasma. Serum albumin (SA) is used as a surrogate marker of nutritional status and inflammation. The prognostic role of SA has been studied in many diseases, including hematologic malignancies. In myelofibrosis (MF), ruxolitinib has been shown to improve SA levels in addition to other metabolic parameters. SA holds particular significance in MF given its ability to capture both nutritional status and inflammation level in a disease hallmarked by hyperactive inflammatory pathways and constitutional symptoms.

Aims: We aim to closely evaluate the significance of SA in MF patients as it pertains to clinical presentation, laboratory correlations, disease genomics, comorbidities and outcomes.

Methods: We retrospectively reviewed an institutional database of 376 MF patients who presented to Moffitt Cancer Center between 1/1/1998 and 12/31/2012 and available SA levels within 30 days of presentation. Laboratory values and prognostic scores were determined at time of first presentation. Overall survival (OS) was measured from time of first presentation until date of death or censored at time of last follow-up. Progression free survival (PFS) was defined as time from first presentation to development of acute myeloid leukemia (AML).

Figure 1.

Results: Our cohort of MF patients had median age of 67 and 69 at diagnosis and presentation, respectively. Most patients had primary MF (73%) with 11% and 16% having post-PV MF and post-ET MF, respectively. First, we looked at the correlation between SA and other clinical factors. SA was positively correlated with hemoglobin (p<0.01) and platelet count (p<0.01), and negatively correlated with age (p<0.01), peripheral blast percentage (p=0.03), ferritin (p<0.01), prognostic scoring models (p<0.01 for IPSS, DIPPS and DIPSS+) and pack-year smoking history (p<0.01). SA did not correlate with spleen size or any specific somatic mutation, but negatively correlated with somatic mutation burden (p=0.03). On univariate regression, SA was associated with inferior PFS (HR: 0.31 [0.13-0.72]; p<0.01) and OS (HR: 0.25 [0.17-0.36]; p<0.01). Four cohorts were created based on SA: cohort I-SA 2.5-3.5 g/dL (n=31); cohort II-SA 3.6-4.0 g/dL (n=98); cohort III-SA 4.1-4.5 g/dL (n=182); and cohort IV-SA=4.5 g/dL (n=84). OS increased with increasing SA with median OS (in months) of 9.34, 25.3, 48.4, and 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 scores (IPSS, DIPSS, DIPSS+) and comorbidities. For PFS, SA remained significant when controlling for IPSS and DIPSS (p=0.01) but lost significance (p=0.08) when controlling for DIPSS+. Multivariable 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, it has independent prognostic value in incorporation into previously existing prognostic systems (IPSS, DIPSS, and DIPSS+). Patients were assigned 0, 2, 3, and 3 points for low (LR), intermediate-1 (int-1), intermediate-2 (int-2), and high risk (HR), respectively. Patients were then assigned -1, 0 and 1 point for SA ≥4.3, 3.8-4.3, and <3.8 g/dL, respectively. Risk groups were defined as LR (<1.0 points), int-1 (1 point), int-2 (2 points), and HR (3-4 points). Survival curves and histograms displayed better capture of LR and HR prognostic groups with median OS similar to standard prognostic modeling (see figure).

Summary/Conclusions: SA level is independently prognostic in MF and correlates with variables known to hold prognostic value. Its representation of nutritional status, inflammation, and comorbidities imbues it with special status in predicting outcome. Its incorporation into known prognostic scoring systems provides an improved ability to accurately capture low and high-risk subgroups.

E1324

CLINICAL UTILITY OF NEXT-GENERATION SEQUENCING IN THE MANAGEMENT OF MYELOPROLIFERATIVE NEOPLASMS

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Background: The new classification of myeloproliferative neoplasms (MPNs) according to World Health Organization (2016) has been developed to improve clinical outcomes and risk stratification of patients. In addition to mutational testing, 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 expanded to high quality exonic nonsynonymous, intronic splice site, frameshift, nonsense and known pathogenic synonymous variants. Variants with global mean allele frequency >1% were identified using multiple population databases (1000 genomes, ESP, ExAC) and excluded. Each patient’s TAR-seq results were reviewed alongside their clinical information systematically by at least two hematologists with expertise in MPN, and disagreements were resolved by consensus.

Results: 179 patients fulfilled the 2008 WHO diagnostic criteria for MPN: 107 were diagnosed with myelofibrosis (MF), 26 with polycythemia vera (PV), 21 with Essential Thrombocythemia (ET) and 32 with unclassifiable MPN. MF was unclassifiable and 12 with MPN/AML. In 6 patients with ‘triple negative’ MPN, who lacked mutations in the driver genes JAK2, CALR and MPL, TAR-seq confirmed the diagnosis of MPN in 13 patients. Variants were subsequently confirmed in 159 patients using a combination of Sanger sequencing, targeted panel and whole exome sequencing. In total, 60 patients were referred for mutation testing and 94 mutational analyses were performed. In 17 patients referred for NGS testing, mutations were identified in both new and known genes. Most mutational events were new and were confirmed in 15 patients. The most frequently mutated genes were JAK2, CALR, MPL and ASXL1. Lastly, given its independent prognostic value, TAR-seq results were used to modify patient management and changed patient care in 11 patients. In 6 patients with triple negative MPN, mutations in the driver genes JAK2, CALR and MPL were also detected. In total, TAR-seq identified 159 patients with MPN, 107 with MF, 26 with PV, 21 with ET, 32 with MPN/AML. In 6 patients with ‘triple negative’ MPN, who lacked mutations in the driver genes JAK2, CALR and MPL, TAR-seq confirmed the diagnosis of MPN in 13 patients. Variants were subsequently confirmed in 159 patients using a combination of Sanger sequencing, targeted panel and whole exome sequencing. In total, 60 patients were referred for mutation testing and 94 mutational analyses were performed. In 17 patients referred for NGS testing, mutations were identified in both new and known genes. Most mutational events were new and were confirmed in 15 patients. The most frequently mutated genes were JAK2, CALR, MPL and ASXL1. Lastly, given its independent prognostic value, TAR-seq results were used to modify patient management and changed patient care in 11 patients. In 6 patients with triple negative MPN, mutations in the driver genes JAK2, CALR and MPL were also detected.
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 comorbidity 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 76.5 years (range 35.6-89.0) with a male prevalence (57.1%); International Prognostic Score System (IPSS) was intermedium-low (37.5% intm-1, 47.5% intm-2, high 18.4%), transfusion dependency and spleen enlargement were present in 23.8% and 74.7% of pts, respectively (62.4% with spleen ≥10 cm). TSS was <20 in 131 pts (38.2%); 62 (18.1%) pts had a BMI<21 (corresponding to lower quartile). CCI was zero in 105 pts (30.6%), one in 74 pts (21.6%), two in 58 pts (16.9%) and ≥3 in 106 pts (30.6%). OS from MF diagnosis was 3.6 yr (range 0.4-28.6) and median RUX exposure was 21.2 months (3-56.2). In multivariable Cox regression analyses of 845 patients with PMF, PET-MF and PPV-MF treated in 35 German hematology centers – a retrospective field study

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Background: Primary myelofibrosis (PMF) as well as secondary post essential thrombocythemia (pet)-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, v-cfemalgia, prognostic factors, past/current treatment and blood count, degree of MF in bone marrow and transfusion frequency was designed. It was distributed to participating centers (n=35, mostly private offices) throughout Germany and analyzed centrally. Time period of collection 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 group 3, only 60.5% of pts in group 1 were at intm-2 IPSS risk, and 48.6% of pts in group 2 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.
CALR Mutation Type Influences the Risk of Thrombosis in Essential Thrombocythemia According to a Cooperative Study Between Two Spanish Centers

Aims: The objective of this study is to define the clinical meaning of CALR mutation type in ET.

Methods: We analyzed 309 ET patients from two hospitals: H.C.U. Santiago and H.U. of Gran Canaria Dr. Negrín. Dates of diagnosis were between 1-11-90 and 1-10-2016, and the median follow up was of 6.88 years. Patients were treated according to local protocols. We collected clinical data of patients at diagnosis and during follow-up as well as events such as thrombosis, transformation to myelofibrosis (MF) or acute leukemia (AL). Thrombosis associated with diagnosis refers to those events happening from two years before to diagnosis until diagnosis. The statistical analyses were performed with R Core Team (2016) and IBM SPSS 21.0.

Results: JAK2V617F mutation was present in 60.5% of the patients, 1.9% had MPL mutations, 14.5% were CALR type-1 like, 11% were CALR type-2 like and 11% were CALR wild type. In three cases, we were not able to classify CALR mutation as type-12 like. With regard to the clinical events: 21 patients (6.8%) had thrombosis associated with diagnosis, and 34 (11%) at least 1 thrombosis per center. Results: Gender was equally distributed (50%/50%). Pts ages at initial diagnosis were as follows:<50 years (y) (11%), 50–60y (19%), 61-70y (31%), and >70y (40%). Current age was >65y in 70% of all pts. PMF represented the largest MF cohort (77%), followed by pet-MF (10%), ptyr-MF (7%) and unspecified (6%). Most pts (75%) had bone marrow fibrosis (10%) and decreased cellularity (36%), <1y (15%), unknown (1%). Key current blood values at time of diagnosis included abnormal thrombocyte counts (<500GPT/L; 6%) <100GPT/L (10%); ≤450GPT (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 ≥10.0 (68%), 8–10.0 (68%), <8.0 (8%) and 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 (DIPPS) score was calculable in 495 pts: 19% low risk, 52% intermediate risk, 23% high risk disease. Concomitant thrombosis 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.

Figure 1.

Summary/Conclusions: The type of driver mutation is associated with a different risk of thrombosis. Among the two types of CALR mutation, patients have similar clinical characteristics except for the risk of thrombosis which seems lower in CALR type-2 like compared to type-1 like. This finding shows the importance of studying the CALR mutation type in ET.

CALR Mutation Type Influences the Risk of Thrombosis in Essential Thrombocytemia According to a Cooperative Study Between Two Spanish Centers

Aims: We prospectively analyzed the levels of leukocyte-platelet (Le-Plt) aggregates, together with levels of soluble selectins, in a group of pts with Ph MPN with diagnosis and during therapy.

Methods: Our study included 90 consecutive de novo Ph MPN pts (37 polycythemia vera, 27 essential thrombocythemia, 26 primary myelofibrosis), diagnosed according to WHO criteria. According to therapy, pts were assigned as: hydroxyurea (HU) 7.8%, aspiron (ASP) 55.6%, hydroxyurea+aspirin (HU+ASP) 31.1%, although 5.6% of pts were without therapy. Neutrophil-platelet (Neu-Plt) and monocyte-platelet (Mo-Plt) aggregates were determined in whole blood samples (EDTA/CTAD) by flow cytometry. Aggregates were estimated as fraction (%) of CD42b+CD61+ neutrophils and monocytes. Plasma levels of E-, L- and P-selectins were determined by enzyme immunoassay. All analyses were performed on 9 pts with thrombosis and 9 pts without thrombosis.

Results: In all pts, mean levels of Neu-Plt and Mo-Plt aggregates at diagnosis were significantly elevated in comparison to control values (22.9% vs 8.9% and 13.0% vs 5.2% respectively, p<0.01). Mean concentration of soluble E-, L- and P-selectins were also significantly higher in Ph MPN than in control group (34.2 ng/mL vs 19.0 ng/mL; 2748.7 ng/mL vs 1322.0 ng/mL and 294.0 ng/mL vs 69.8 ng/mL, respectively, p<0.01). Mean levels of Neu-Plt and Mo-Plt aggregates in response to therapy were significantly reduced compared to baseline levels (Figure). Significant reductions were observed for E-selectin levels in HU+ASP group, for L-selectin levels in all three therapy groups and for P-selectin levels in HU and HU+ASP groups (Table). During the median follow up of 39 months from diagnosis of Ph MPN, thromboembolic events occurred in 13.3% of pts (12/90), particularly: 0/7 on HU, 3/50 on ASP, and 9/28 on HU+ASP. In this subgroup we observed increased baseline levels of Neu-Plt and/or Mo-Plt aggregates in 9/12 pts, while all 12 pts had increased at least one soluble selectin, predominantly P-selectin. Resting revealed that all 9 pts with thrombosis and increased aggregates level at baseline, normalized those levels after therapy, while only 4/12 pts normalized soluble selectin levels.
HEAT SHOCK PROTEIN 27 EXPRESSION IS INCREASED IN PATIENTS WITH PRIMARY AND SECONDARY MYELOFIBROSIS AND MAY BE AFFECTING THEIR SURVIVAL


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, the Spearman rank correlation, the log-rank test and the Cox regression analysis were used, cut-off point for survival analyses was determined using the ROC curve analysis.

Results: Relative expression of HSPB1 differed significantly between diagnoses (P=0.011); it was significantly higher in patients with PMF and SMF than in control group (P=0.05 for both comparisons), but did not differ between PMF and SMF patients (non significant). Increased expression was associated with increase in the spleen size (P=0.009) and JAK2 V617F mutation (P=0.073). We did not detect significant associations with other disease specific features. Lower HSPB1 expression was associated with inferior overall survival in both univariate (HR 3.2; P=0.04) and multivariate analysis (HR 6.12; P=0.034) 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).

Summary/Conclusions: We have found elevation of blood and endothelial cell activation markers at baseline in Ph-MPN. Cytoreductive and antiaggregatory therapy reduced the mean level of Le-Plt aggregates and concentration of soluble selectins. In subset of pts with thrombosis, therapy lead to normalization of Le-Plt aggregate levels, with incompletely normalized soluble selectin levels. Even with normal Le-Plt aggregates, observed elevated selectin levels can explain persistent thrombotic risk due to intrinsic changes in relationship between blood and endothelial cells as a part of biology of Ph-MPN itself.

E1329

NON-DRIVER MUTATIONS IDENTIFIED BY A 190-GENE NEXT GENERATION SEQUENCING PANEL IN PATIENTS WITH PRIMARY MYELOFIBROSIS AND POST-POLYCYTHEMIC/ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS

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Background: It is a consensus that the driver mutation is an independent prognostic factor in PMFs. Moreover, some non-driver mutations are found associated with initiation, progression and prognosis in PMFs. However, a recent study from the AGIMM (AIRC- Gruppo Italiano Malattie Mieloproliferative) group showed that the type of driver mutation did not influence prognosis in post-PV/ET MF. These observations proved that there were indeed some differences in these two types of MF.

Aims: The aim of current study was to describe the non-driver mutation landscape and the molecular differences between the patients with PMF and those with post-PV/ET MF.

Methods: Targeted gene sequencing was carried out at diagnosis. We sequenced 190 genes across 62 patients, resulting in 229 high-confidence mutations. The average gene coverage was 99%. The average read depth was 540×. Also, 92% of targeted regions were covered with >20×. Every mutation identified in this study was then compared against these expected patterns and categorized into “oncogenic,” “possible oncogenic variants,” or “unknown significance”. Using copy number-adjusted VAF, we reconstituted 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=0.865) and PMF patients vs. without driver mutations (3 vs. 3.18, P=0.668). In PMFs, 12 non-driver genes were mutated in >5% of patients, namely ASXL1 33.3%, U2AF1 22.2%, TET2 15.6%, FAT1 15.6%, SETBP1 13.3%, SRSF2 8.9%, CUX1 8.9%, EP300 8.9%, FAT2 6.7%, NOTCH3 6.7%, EZH2 6.7%, and GATA3 6.7%. In post-PV/ET MFs, ASXL1 (41.2%) was the most frequent mutation, followed by TET2 (29.4%). U2AF1 and SRSF2 mutations were significantly more frequent in PMF than in post-PV/ET MF. Moreover, SETBP1 and FAT1 were mutated in PMF more often and not mutated in post-PV/ET MF. Figure 1A-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 1D.
Baseline TSS, a 3-level grouping factor for GH/QOL change (deterioration vs stable vs improvement), and an interaction term between baseline TSS and the GH/QOL grouping factor.

Results: 301 patients randomized to ruxolitinib [N=149] or placebo [N=152]
completed TSS at baseline (45% female, median age 68 [range 40-91]). Median baseline TSS was 16.8 (range 0 to 52.7). Pooled SD at baseline and week 24 in TSS was 11.4 and 11.6, respectively, resulting in estimated meaningful changes of 3.6-5.8 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 (27%) 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 (-3.8 to 0.8), and -6.8 (-9.0 to -4.6), and for percent changes 20% (-6% to 46%), 17% (-11% to 44%) and -34% (-45% to -22%). ANCOVA revealed that baseline TSS statistically significantly impacted p=0.02. Figure 1 demonstrates how group change in 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 percent of 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. Survival was improved 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 was reported in response to ruxolitinib was reported. This results may be partially explained by the selection of patients with endogenous erythropoietin level below 250 IU/l, but they could also suggest synergistic activity of ESA and ruxolitinib.

E1333 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)-1– 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 (≥25 cm). Starting dose was based on baseline platelet (PLT) count (<200×109/L), platelet count (<50×109/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%, 85%). 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 (20%, 11%, 23%), but these rarely led to discontinuation. Overall rates of nonhematologic grade 3/4 AEs were <2%, except for pneumonia (4.5%), pyrexia (2.5%), asthenia (2.2%), and dyspnea (2.2%). Infections in ≥5% of pts were pneumonia infection (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 ≥50% reductions. Median 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 (2.5–7.5 wk), 5.3 wk (2.6–8.0 wk), and 8.1 wk (3.1–72.3 wk). From wk 4 to 48, 39%, 43%, 41%–44%, and 48%, and 48% of pts achieved a clinically meaningful response on the FACT-Lym TS: proportions of responders on the FACT-Fatigue were 42%–49%, 46%–49%, and 55%–61%.

Summary/Conclusions: RUX was safe and generally well tolerated. Interestingly, lower-risk pts received higher starting doses yet had lower rates of hematologic AEs. Additionally, lower-risk pts remained on treatment longer than higher-risk pts, with fewer discontinuations due to AEs. Lower-risk pts also achieved slightly better spleen size reductions and symptom improvement than higher-risk pts, suggesting that earlier RUX treatment may lead to greater benefits in pts with MF.

E1334 SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS WITH MYELOFIBROSIS (MF) WHO STARTED TREATMENT AT 10mg BID AND HAD THE DOSE UPTITRATED IN THE PHASE 3B EXPANDED-ACCESS JUMP STUDY


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Background: The most common hematologic grade 3/4 AEs were anemia (22%, 44%, 55%) or 15mg bid (26%, 32%, 33%). Median exposure was 16, 11, and 14.5 cm) were similar in all 3 groups. Most pts started at 20mg bid (68%, 57%, 59%) or 15mg bid (20%, 11%, 23%), but these rarely led to discontinuation. Overall rates of nonhematologic grade 3/4 AEs were <2%, except for pneumonia (4.5%), pyrexia (2.5%), asthenia (2.2%), and dyspnea (2.2%). Infections in ≥5% of pts were pneumonia infection (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 ≥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 (2.5–7.5 wk), 5.3 wk (2.6–8.0 wk), and 8.1 wk (3.1–72.3 wk). From wk 4 to 48, 39%, 43%, 41%–44%, and 48%, and 48% of pts achieved a clinically meaningful response on the FACT-Lym TS: proportions of responders on the FACT-Fatigue were 42%–49%, 46%–49%, and 55%–61%.

Figure 1.
Methods: in pts with MF. The presence of a subset of JUMP pts provides information on this approach. Further evidence from clinical practice suggests that starting RUX at 10 mg bid and subsequently titrating may reduce the risk of cytopenia development. An ad hoc analysis of a subset of JUMP pts provides information on this approach.

Aims: To assess the safety and efficacy of RUX at a starting dose of 10 mg bid in pts with MF.

Methods: Pts with high-, Int-2-, or Int-1–risk MF were eligible. Int-1–risk pts had a palpable (≥25 cm) spleen. Protocol starting doses (5, 15, or 20 mg bid) were based on baseline platelet (PLT) counts (<50 to <100×10^9/L, 100 to 200×10^9/L, >200×10^9/L, respectively). Although not per protocol, some pts started RUX at 10 mg 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), 112.1 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 time to RUX was 25.8 mg/d (SD, 10.1) and was comparable to those (33.2 and 23.3 mg/d) of patients starting at higher doses (20 and 15 mg bid).

The most common hematologic grade 3/4 AEs were anemia (27.1%; overall, 34.1%) and thrombocytopenia (14.6%; overall, 16.3%). Hb and PLT dynamics were also similar. AEs (all-grade [grade 3/4]) in >10% of pts included pyrexia (14.6% [4.2%]), asthenia (12.5% [0%]), weight increase (12.5% [0%]), abdominal pain (10.4% [0%]), headache (10.4% [2.1%]), and peripheral edema (10.4% [0%]). Infections in >2 pts included herpes zoster (8.3%), gastroenteritis, nasopharyngitis, and septic shock (6.2% each). At wk 24, 69.9% of pts (14/23) had a ≥50% reduction from baseline in spleen length and 26.1% (6/23) had ≥50% reductions; rates were similar at wk 48 (58.3% [7/12] and 25.0% [3/12]). Most pts (56.3%) achieved a ≥50% reduction at any time. Pts also experienced significant improvements in symptoms. From wk 4 to 48, 43%-59% (3/12) experienced significant improvements in symptoms.

Summary/Conclusions: A small cohort of pts in JUMP started at 10 mg bid, and had the dose uptitrated during the first 8 wk 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 (NCT029666353).

E1335

HYDROXYUREA IS ASSOCIATED WITH SKIN TOXICITY IN MYELOPROLIFERATIVE NEOPLASMS: RESULTS FROM A PROSPECTIVE NON-INTERVENTIONAL STUDY

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Background: Until today, hydroxyurea (HU) remains the most commonly used cytoreductive drug in patients (pts) with classic myeloproliferative neoplasms (MPN), i.e. essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF). However, mucosal lesions, cutaneous ulcers, and pre-carcinomatous skin alterations such as actinic keratoses are being considered as potential side effects of HU.

Aims: We sought to investigate the occurrence of skin toxicity in MPN pts under HU compared to other (non-HU) cytoreductive drugs in routine clinical practice.

Methods: Classic MPN pts regularly presenting at the outpatient centers of the University Hospital of Ulm and Johannes Wesling Clinic Minden were included in our non-interventional study after having given informed consent. Skin alterations were evaluated prospectively between December 2010 and November 2016.

Results: In total, 151 MPN pts under cytoreductive therapy were included (ET, n=55; PV, n=55; MF, n=41). Primary MPN diagnosis was made between 1979 and 2012 at a median age of 55 years (range, 22-82). Median duration of the disease at the 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 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 (basal cell carcinoma, n=3; malignant melanoma, n=1). Although pts of the HU cohort were exposed longer to the drug compared to pts of the non-HU group, numbers of skin events in non-HU treated pts were as following: n=5 under IFN-a (local reaction after subcutaneous injection, n=3; actinic keratoses, n=3), dry skin / xerostomia (n=2). Of note, four malignant skin diseases were reported under HU therapy (basal cell carcinoma, n=3; malignant melanoma, n=1). All pts of the HU cohort were exposed longer to the drug compared to pts of the non-HU group, numbers of skin events in non-HU treated pts were as following: n=5 under IFN-a (local reaction after subcutaneous injection, n=3; actinic keratoses, n=1), and none under ruxolitinib. In 3/126 (2%) non-HU treated pts, occurrence of skin toxicity led to discontinuation of the corresponding cytoreductive drug. Interestingly, both skin ulcers as well as the single events ‘basal cell carcinoma’ and ‘actinic keratosis’ occurred under combination therapy with HU. Taken together, skin alterations occurred more frequently under HU compared to non-HU treatment (52/120 [43%] vs 9/126 [7%]; p=0.0001), and the same was true for treatment discontinuations due to skin toxicity (16/120 [13%] vs 3/126 [2%]; p=0.014).

Summary/Conclusions: According to our prospective observation, skin toxicity was clearly associated with HU treatment compared to other cytoreductive drugs. This resulted in a higher rate of HU treatment termination due to skin toxicity. However, median exposure time to HU was clearly associated with HU treatment compared to other cytoreductive drugs.

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-...
negative. Median age at BP was 71.3 years (range 46.3-86), being higher in PV (median 73 years, range 46.3-84.7) compared to ET (median 66.8 years, range 54.4-86, P = 0.0318) and PMF (median 67.9 years, range 48.1-84.9, P = 0.016). The complete blood count at leukemic evolution was not influenced by the initial diagnosis. At the time of BP, 31 out of 44 patients (70%) for whom cytogenetic analysis was available showed an abnormal karyotype (22 patients with complex karyotype or high risk aberrations). JAK2 mutated MPN can evolve into JAK2 wild type AML (9 of 28 patient with blasts DNA available), while CALR mutation was identified also in AML blasts in all 6 patients for which DNA was available. Time to leukemic evolution was shorter in PMF (35.3 months, range 3.6-141.1) compared to ET (176.7 months, range 14.4-362.3, P <0.001) and PV (129.1 months, range 17-367.8, P<0.001). According to chronic phase driver mutation, time to leukemic evolution was shorter in JAK2 V617F mutated PMF compared to CALR mutated PMF (30.6 vs 138 months, P =0.024), but not statistically different in JAK2 mutated ET compared to CALR mutated ET (123.4 vs 203.2 months, P =0.121). Outcome was dismal, independently from the diagnosis at BP: overall survival (OS) in PMF patients with monocytosis was 27.3 months, and for patients with eosinophilia the OS was 28.5 months, compared to 46.4 months for patients under the cut-off. Considering the whole cohort, 61.8% of patients had normal monocytosis, 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 27.9 vs 64.4 months. Progression-free survivals were not calculated, since only 2 patients had BM- or PB-documented progression during follow-up.

Summary/Conclusions: We observed that the presence of monocytosis at diagnosis in PMF was associated with a halving of the median OS, while eosinophilia decreased the median survival to one-fifth; basophilia also associated with a reduction in survival, of approximately 20%. The application of specific cut-offs calculated for the cohort improved the differentiation and stratification of patients, with moderate to high specificity, further clarifying the negative prognostic impact of these three variables, at diagnosis, in PMF. Our results show that even simple, inexpensive and readily available parameters can be used to predict survival in PMF patients, and suggest that their integration into established scores could further increase the prognostic accuracy of the latter.

E1337 BLAST PHASE IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A SINGLE INSTITUTION RETROSPECTIVE ANALYSIS OF 85 PATIENTS

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Background: Classic Ph-negative myeloproliferative neoplasms (MPN) include essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). Chronic evolution can lead MPN patients in chronic phase (CP) to develop acute myeloid leukemia (AML), called blast phase (BP); this event occurs at rates of approximately 1% in ET, 4% in PV and 20% in PMF over the first decade from MPN diagnosis.

Aims: To evaluate differences in clinical features and outcome in 85 patients with Ph-negative BP who presented with monocytosis (AC >0.75 G/L), basophilia (AC >0.2 G/L) and eosinophilia (AC>0.6 G/L) in BM and peripheral blood (PB) analyses dating from the time of diagnosis, and have complete charts with no missing data. After the exclusion of reactive causes, monocytosis was defined as an absolute count (AC) >1.0 G/L, eosinophilia as an AC >0.6 G/L and basophilia as an AC >0.2 G/L.

Results: We studied 55 evaluable patients (73% male) with a median age at diagnosis of 70.1±11.7 years old. At diagnosis, 20% of patients had monocytosis, with no significant differences according to gender or age. The median overall survival (OS) in PMN patients with monocytosis was 27.3 months, and two-thirds of these patients with monocytosis had a cut-off of 0.75 G/L. The reason of death was not available, but they presented with Ph-negative MPN. The median survival in Ph-negative ET patients of whom 15 presented with Ph-negative MPN was 85 months who progressed to BP, with a known monocytosis cut-off of 0.75 G/L. Patients with monocytosis had a median OS of 27.9 months, compared to 64.4 months for patients under the cut-off. We identified 12.7% of patients with eosinophilia at diagnosis, with no differences according to gender or age. PMF patients with eosinophilia had a five-fold lower median OS compared with patients without (6.1 vs 32.5 months, respectively). We obtained a new cut-off of 0.25 G/L of eosinophils, which separated patients with a specificity of 77.8% (95% CI: 57.7-91.4%), 29.1% of patients had an eosinophil AC above the cut-off, with a median OS of 17.3 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 basophil, with a specificity of 88.9% (95% CI: 70.8-97.6%), 20.0% of patients had an 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 monocytosis, 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 27.9 vs 64.4 months. Progression-free survivals were not calculated, since only 2 patients had BM- or PB-documented progression during follow-up.

Summary/Conclusions: We observed that the presence of monocytosis at diagnosis in PMF was associated with a halving of the median OS, while eosinophilia decreased the median survival to one-fifth; basophilia also associated with a reduction in survival, of approximately 20%. The application of specific cut-offs calculated for the cohort improved the differentiation and stratification of patients, with moderate to high specificity, further clarifying the negative prognostic impact of these three variables, at diagnosis, in PMF. Our results show that even simple, inexpensive and readily available parameters can be used to predict survival in PMF patients, and suggest that their integration into established scores could further increase the prognostic accuracy of the latter.

E1338 TELOMERE LENGTH IS REDUCED IN ESSENTIAL THROMBOCYTHAEMIA PATIENTS COMPARED TO AGE AND GENDER MATCHED HEALTHY CONTROLS

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Background: Essential thrombocythaemia (ET) is a clonal stem cell disorder, commonly diagnosed in the 6th or 7th decade of life. ET is associated with risk of thromboembolic events, hemorrhage, constitutional symptoms, progression to myelofibrosis and acute myeloid leukemia. In over 85% of patients a clonal driver can be identified with mutations in JAK2 (50-60%), Caletriculin (CALR) (25-30%) or the thrombopoietin receptor (MPL) (3-5%); the remainder of patients are termed "triple negative" (TN). Telomeres are non-coding regions of DNA consisting of thousands of repeated sequences (TTAGGG) and are considered central to chromosomal integrity and genomic stability. In healthy adults, telomere length (TL) progressively shortens with age; therefore, TL is considered as a marker of aging and genomic stability. Telomerase activity in several hematological malignancies have been shown to be characterized by shortened TL.

Aims: Determine if there is TL shortening in patients with ET when compared to age and gender matched controls and establish the effects of cyto-reductive treatment.

Methods: 100 patients were included in the study (27 with CALR, 35 JAK2V617F and two MPL515W mutations. 36 patients were TN). Most patients were female (70% 70/100); median age was 45 years (range 20 - 86 years).
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 vs healthy controls, have shortened TL. This shortening is more pronounced in HC treated patients indicating that there may be a therapy effect as has been observed after HC treatment in sickle cell disease. Of note IFN treated patients had more normal TL suggesting that the disease related TL effects may be reversed by this agent.

E1339
NUTRITION IN MYELOFIBROSIS: CORRELATES FROM THE COMFORT-1 STUDY
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Methods: Data from the COMFORT-1 trial of ruxolitinib versus placebo was obtained from the Incyte for independent analysis. Data was analyzed for correlations with symptom burden and survival along with other variables. Symptom burden was assessed by the MF-SAF v2.0 (Mesa et al. Blood 2008;112(11):5224). MF also leads to deficient LDL and cholesterol levels compared to age matched controls (Mesa R A et al. Blood 2007;110(11):2548). Both hypocholesterolemia (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: From the COMFORT-1 trial of ruxolitinib versus placebo cohort; for the 18 patients, whose TL was below the first centile, 55% (10/18) were CALR positive, p=0.0009; JAK2V617F positive patients (8/18) had prior exposure to HC; 34/100 were not on cytoreductive therapy. Remaining treatments were ruxolitinib (5), busulphan (4), andanagrelide (1) and vorinostat (1). Independent of mutational status there was significant TL shortening in untreated patients, p=0.05; however, upon evaluating the impact of cytoreductive therapy on TL we noted that ET patients currently on IFN but 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 shortened TL.

Summary/Conclusions: We document for the first time that ET patients, when compared to age and gender matched healthy controls, have shortened TL. This shortening is more pronounced in CALR and JAK2V617F positive patients. Concerning therapy whilst present in untreated patients TL shortening was more pronounced in HC treated patients indicating that there may be a therapy effect as has been observed after HC treatment in sickle cell disease. Of note IFN treated patients had more normal TL suggesting that the disease related TL effects may be reversed by this agent.

E1340

Background: To evaluate the prognosis of patients with Essential Thrombocytemia (ET) 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 cytodestructive 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, hopely, the introduction of new drugs as JAK-2 inhibitors could change the prognosis of ET patients.

**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%), actinic keratosis (39.7%), hyperpigmentation of the skin (23.8%), pruritus (23.8%) and non-melanoma skin cancer (22.2%). In figure 1 we detail all the skin alterations that we have found.

**Summary/Conclusions:** Cutaneous involvement in MPNs is more frequent than expected and it is usually underdiagnosed. Some of these lesions could be prevented with the correct treatment of their pathology and adequate 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 cytodestructive therapy.

**E1342**

**HEMOGLOBIN AND WHITE CELL COUNT IN PATIENTS CLINICALLY SUSPECTED TO HAVE ESSENTIAL THROMBOCYTHEMIA MAY HELP IN PREDICTING EARLY PRIMARY MYELOFIROSIS OR UNCLASSIFIABLE MYELOPROLIFERATIVE NEOPLASM**

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**Background:** Classification of myeloproliferative neoplasms (MPN) in patients presenting with thrombocytosis can be challenging. Relying only on clinical features may lead to misclassification of patients in the early stages of primary myelofibrosis (PMF) as essential thrombocythemia (ET). Although bone marrow (BM) biopsy examination is the gold standard necessary for accurate classification, in clinical practice it might be helpful to identify among patients with a working diagnosis of ET those most likely to have early PMF or unclassifiable MPN (MPN-U). To this end, Carobbio et al. (Am J Hematol. 2012;87:203-4) developed a simple algorithm based on presence of anemia (hemoglobin <120 g/L for females and <130g/L males) and/or leukocytosis (leukocytes ≥13×10^9/L) or elevated LDH (>200 mU/mL). For an accurate classification of the clinical and laboratory features need to be correlated with BM findings, thus collaboration between hematologists and pathologists is essential.

**Aims:** To examine applicability of the Carobbio algorithm in routine practice and its potential use in identifying among patients presenting with thrombocytosis and clinically suspected to have ET, those with early PMF or MPN-U. To identify unfit needs in the diagnosis of MPNs in daily practice upon which further educational initiatives can be built which stress the importance of hematologist-pathologist collaboration.
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 SM by biopsy collected within a year of presentation with thrombocytosis if they presented more than 5 years ago. Out of 122 patients, 48 met the hemoglobin and/or leukocyte criteria outlined in the Carobbio algorithm, Figure. The BM examination was performed on 33 patients who met pre-specified criteria for the timing of bone marrow biopsy. About one third of the 33 patients were not classified for ET or one third for PMF. While 2 of the remaining patients met criteria for PV, the rest were uncertain whether to represent true ET or early PMF, i.e. represented MPN-U (Figure 1).

Summary/Conclusions: Despite its methodological limitations, this initiative confirms that in real world clinical practice the Carobbio algorithm can be used to identify patients with early PMF and MPN unclassifiable among clinical scenarios currently 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 further 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 II STUDY


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Background: MF is a life-threatening hematologic malignancy characterized by splenomegaly and debilitating constitutional symptoms. At the present, the JAK1/2 inhibitors ruxolitinib, only therapeutic option (pts) with hematologic response, has garnered regulatory approval. Although ruxolitinib has been shown to reduce splenomegaly and symptoms in pts with MF, it is associated with dose-limiting cytopenias, and not indicated for pts with platelets <50,000/µL. PAC is an oral kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R. Using data from prospective, open-label studies, population PK modeling and simulations predicted that BID dosing would result in higher steady-state AUC and lower Cmax from early-phase PAC studies, population PK modeling and simulations predicted that BID dosing would result in higher steady-state AUC and lower Cmax from early-phase PAC studies, population PK modeling and simulations predicted that BID dosing would result in higher steady-state AUC and lower Cmax from steady-state PAC trials.

Methods: In total, PK samples were collected up to week 24 from 144 PAC-pts for PK analysis only at weeks 12 and 24 (pre-dose). At the remaining sites, blood samples were collected from PAC-treated pts for PK and PD analysis at a prespecified subset of trial sites. Blood samples were collected on day 1 of week 1 (4 h post-dose), week 3 (pre-dose and 4 h post-dose), week 12 (pre-dose), and week 24 (pre-dose). At the remaining sites, blood samples were collected from PAC-treated pts for PK analysis only at weeks 12 and 24 (pre-dose).

Results: Blood samples were collected up week 24 from 144 PAC-treated pts (78 BID, 64 QD). The PK of PAC was described by a 2-compartment model with first order absorption, first order elimination from the central compartment, and an absorption lag time. PAC QD was associated with higher Cmax and lower Cmin vs PAC BID (Table). Median PAC plasma concentrations during weeks 12 to 24 with QD were similar to those with BID, whereas Cmin (Cminss) at weeks 12 and 24 were higher vs QD dosing by 10% and 15%, respectively. Also, median observed steady-state 4h concentration at week 3 (coincides with Cminss) was 12% higher with QD vs BID dosing.

Summary/Conclusions: As predicted by PK modeling and simulations analyzing PAC 400mg QD was associated with higher Cmax and lower Cmin vs PAC 200mg BID in pts with MF from the PERSIST-2 trial. These differences appear to translate into an improved benefit/risk profile of PAC BID vs QD regimens.

E1344
ZMYM2-FLT3 IS A RARE, RECURRENT, CYTOGENICALLY CRYPTIC FUSION IN MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA THAT IS RESPONSIVE TO FLT3 INHIBITION

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Background: Myeloid/lymphoid neoplasms with eosinophilia are characterised by diverse tyrosine kinase (TK) fusion genes, many of which can be effectively targeted by small molecule inhibitors. More than 70 TK 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 myeloid/lymphoid neoplasms with eosinophilia (MPN-eo) remains unexplained. We hypothesized that some MPN-eo cases may be driven by hitherto undetected cryptic TK fusion genes.

Methods: To screen cases with MPN-eo for TK fusion genes and evaluate the significance of any novel fusions

Results: Of 20 cases tested by RNAseq analysis, just one cryptic TK fusion was identified: ZMYM2-FLT3, predicted to arise as a consequence of an 8Mb inversion at 13q12. Unusually, both breakpoints fell within exons (ZMYM2 exon 20 and FLT3 exon 14, respectively) resulting in an in frame fusion. To 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 (30x10^9/L), eosinophilia (2x10^9/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. Subsequent FISH analysis identified a normal karyotype, and no pathogenetically relevant mutations were identified by a myeloid NGS panel (28 genes). After 10 months, she progressed to myeloid blast phase. Therefore the disease was resistant to AML-induction chemotherapy (FLAG-Ida), an allo-HSCT was performed. At 12 months after allogeneic PBSCT. The ZMYM2-FLT3 fusion gene was identified post mortem. Case 2, a 47 year old male, presented with eosinophilia (4.7x10^9/L), 47% elevated serum tryptase (42µg/l) and a hypercellular BM. Cyto genetics was normal and FIP1L1-PDGFRα, KIT D816V and JAK2 V617F were all negative. There was no response on steroids or hydroxyurea. Following the finding of ZMYM2-FLT3 positivity, treatment with sunitinib was commenced. Blood counts started to improve from day 4 and normalized after 3 weeks. During a pause of 3 weeks due to pulmonary infection, leukocytes/eosinophils rapidly increased, but normalized again within weeks after restart of sunitinib. The patient has been maintained on sunitinib for 10 months (since re-start) and remains in complete hematologic remission.

Summary/Conclusions: ZMYM2 is the fourth gene reported to fuse to FLT3 in myeloid neoplasms but the first FLT3 fusion that is cytogenetically cryptic. Given ZMYM2-FLT3 is a rare, recurrent fusion in myeloid neoplasms with eosinophilia that is potentially responsive to FLT3 inhibition, these findings will become the method of choice to detect rare TK fusions.
**E1345**

**COMPLETE HEMATOLOGIC AND CYTODERGIC RESPONSE IN A PATIENT WITH FIBROBLAST GROWTH FACTOR RECEPTOR 1 ACTIVATED MYELOPROLIFERATIVE NEOPLASM RECEIVING INCB054828**

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Background: Fibroblast Growth Factor Receptor (FGFR) inhibitors have demonstrated efficacy in solid tumors with FGFR pathway activation. INCB054828, a novel, highly selective FGFR1, FGFR2, and FGFR3 inhibitor, is being assessed for the treatment of several advanced malignancies (AACR 2015; Abstract 77). 8p11 myeloproliferative syndrome is an aggressive myeloproliferative neoplasm (MPN) associated with FGFR1 translocation on chromosomes 8p11.

Aims: To describe the characteristics of a patient with FGFR1 activated MPN who achieved a complete hematologic and cytogenetic response with INCB054828 in an ongoing phase 1/2 trial (NCT02393248).

Methods: In this 3-part, phase 1 dose-escalation and expansion trial, eligible adults had any advanced solid tumor (parts 1 and 3) or malignancy with FGFRs/FGFR alteration (part 2). Patients had Eastern Cooperative Oncology Group performance status score ≤1 (part 1) or ≤2 (parts 2 and 3), and were refractory to prior therapy with no known effective standard therapy available to them. Patients received INCB054828 orally on a 21-day cycle (2-weeks on/1-week off) starting at 9mg QD and increasing to 13.5mg QD.

Results: This 51-year-old male patient with 8p11 translocated MPN diagnosis (currently the only patient with MPN enrolled in this trial), presented with abnormal white blood cell (WBC) count (eosinophils, 15%; peripheral blood [PB] blasts, 4%) and abnormal platelet count (68 x 10^9/L). The patient had prior therapy with hydroxyurea. Bone marrow (BM) biopsy at study entry showed 95% cellular marrow, 5% damaged megakaryocytes (8/9,11/2,0,13) in 15% of 20 metaphases, and European Myelofibrosis Network grade MF-1. After 6 weeks of treatment with INCB054828 at a dose of 9mg QD in part 2 of the study, WBC count normalized with disappearance of eosinophilia and PB blasts. BM biopsy demonstrated a normalization of bone marrow differential with 50% cellularity, 1% BM blasts, adequate trilineage hematopoiesis, MF-1 fibrosis, and a complete cytogenetic response. After 4 months of treatment the patient was hospitalized for pneumonia and study treatment was held. The patient progressed to AML shortly after therapy interruption, with BM blasts increasing to 83% and evidence of clonal evolution (47,XY:+8,8(9,11;2;33) [3]/48 idem, +19 [17]). The patient was taken off study at this time (end of cycle 6) and subsequently achieved a complete remission on intensive chemotherapy with fludarabine, cytarabine, idarubicin, and allogeneic BM transplantation. The patient is currently alive and in complete remission.

Summary/Conclusions: INCB054828 showed efficacy in this patient with FGFR1 activated MPN using a 21-day (2-weeks on/1-week off) regimen. Continuous treatment may be necessary to sustain response and avoid rebound as has been seen with other kinase inhibitor therapies. A phase 2 trial has been initiated to evaluate INCB054828 in patients with myeloid/lymphoid neoplasms with FGFR1 rearrangement (NCT03011372).

**E1346**

**THE GRADE OF STROMAL CHANGES IMPACTS ON PROGNOSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS**

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Background: Recently, a detailed grading system for the assessment of bone marrow stromal changes has been proposed in primary myelofibrosis, proved as follows: bone marrow fibrosis: MF-0 in 9 cases, MF-1 in 60, MF-2 in 31 and MF-3 in 22; collagen deposition: Co-0 in 64 cases, Co-1 in 23, Co-2 in 21 and Co-3 in 14; osteosclerosis: Ost-0 in 72 cases, Ost-1 in 24, Ost-2 in 19 and Ost-3 in 7. Patients’ population was composed of 56 males and 66 females (MF-0=1/2) with a median age at diagnosis of 68 years (range 30-85). Clinically, at presentation, anemia was always present in 20 (16%) patients, leukocytosis more than 25 x10^9/L was identifiable in 4 (3%) patients, and platelets count less than 100 x10^9/L in 7 (6%) cases. AKT2617F mutation was detected in 81 cases (66%). Among the remaining 41 JAK2-negative patients, 4 and 27 carried MPL and CALR mutations, respectively; 10 out of 122 resulted “triple-negative”. According to the International Prognostic Scoring System, 38 cases were stratified as low risk, 51 as intermediate-1 risk, 21 as intermediate-2 risk, and the remaining 12 as high risk. By the time of the analysis, 21 (17%) patients had died: leukemic evolution occurred in 14 (11.5%) patients, whereas thrombotic or hemorrhagic events occurred in 25 (20.5%). Subsequently, a comprehensive grade of bone marrow stromal changes ranging from 0 to 9 allows us to distinguish 68 (72%) cases with low-grade stromal changes (total score: 0-4) and 34 (28%) with high-grade stromal changes (total score: 5-9). Clinically, patients with high-grade stromal changes presented more frequently with anemia, thrombocytopenia, leukocytosis, peripheral blood blasts and increased lactate dehydrogenase levels. The grade of bone marrow stromal changes resulted strictly associated with the International Prognostic Scoring System and the overall mortality (low-grade: 10 dead out of 88 vs high-grade: 11 dead patients out of 34; p=0.013). Finally, the grade of bone marrow stromal changes was effective in discriminating the overall survival of the patients with low-grade and high-grade stromal changes (Log-Rank test: p=0.0002).

Summary/Conclusions: A detailed evaluation of the bone marrow stromal changes has important prognostic implications and can be used at diagnosis in the clinical stratification of the patients affected by primary myelofibrosis. Further studies are needed to test if the prognostic significance of this grading system remains during the follow-up.

**E1347**

**INCREASED RISK OF INFLAMMATORY BOWEL DISEASE IN PATIENTS WITH PHILADELPHIA NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS**

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Background: Studies reveal that patients with inflammatory bowel disease (IBD) may have increased risk of haematological cancers. Moreover, Philadelphia negative chronic myeloproliferative neoplasms (MPNs) have previously been associated with autoimmune diseases, including IBD. Nevertheless, to our knowledge, the risk of IBD has not been investigated in patients with MPN.

Aims: We undertook a nationwide population-based matched cohort study, and estimated the risk of the MPN-IBD association.

Methods: We used valid Danish national registries, covering more than 5 million individuals, and included all patients diagnosed with either essential thrombocythemia (ET), polycythemia vera (PV), myelofibrosis (MF), or unclassifiable myeloproliferative neoplasm (MPN-U).

Results: INCB054828 showed efficacy in this patient with FGFR1 activated MPN using a 21-day (2-weeks on/1-week off) regimen. Continuous treatment may be necessary to sustain response and avoid rebound as has been seen with other kinase inhibitor therapies. A phase 2 trial has been initiated to evaluate INCB054828 in patients with myeloid/lymphoid neoplasms with FGFR1 rearrangement (NCT03011372).
ESSENTIAL THROMBOCYTHEMIA WITH AQUAGENIC PRURITUS: AN ENTITY WITH MORE AGRессIVE CLINICAL AND BIOLOGICAL PROFILE AT THE DIAGNOSIS AND A HIGH MORBIDITY DURING THE FOLLOW-UP
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Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are Ph-negative myeloproliferative neoplasms in which arterial or venous thromboses and phenotypical evolutions (leukemia, myelofibrosis) are the most recurrent complications. Aqueagenic 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 tested for isotopic red mass cells if appropriate.

Results: Among the 396 ET patients, 42 (10.6%) suffered from AP. Interestingly, the median age at diagnosis of these patients was lower (51.6 vs 63.8%, p<0.0001). Furthermore, they presented more symptoms as erythrocytosis, hyperviscosity, constitutional symptoms and splenomegaly (p<0.01). ET patients with AP were more proliferative (more polycythemic but less thrombo-
cythemic, p<0.005), 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 throm-
bolic 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 thrombosis was also different with 50/50 vs 2/3:1. 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 also had higher risk of thromboses and phenotypic evolutions than ET without AP. Despite that these patients have a higher overall survival. So, the presence of AP in ET with ET characterizes 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: Anagrelide is a useful drug in the control of thrombocytopathy in MPN. Although it is known that in therapeutic levels it primarily influences in the post-mitotic phase of megakaryocytic development interfering with its complete maturation, its mechanism of action is still unknown.

Aims: The progress in the diagnosis of MPN due to the discovery of driver mutations (JAK2, calreticulin and MPL) leads us in the present study to correlate them with the response to anagrelide in a group of patients treated with this drug, investigating the possible interference in the referred biological pathways.

Methods: The medical records of patients treated with anagrelide in our centre between 1993 and 2015 were studied. The median age was 49 years, with 19 patients older than 60 years. 83% were female and 17% were male. The diagnosis was initially carried out based on the WHO criteria 2008 and subsequently reviewed the medical records with the new criteria of 2016. A molecular study on peripheral blood samples was carried out using quantitative allelic-specific PCR techni-
quies for JAK2, qualitative for MPL (L515V mutation) and Sanger sequencing of exon 9 for calreticulin. Type 1 mutation was considered at 52 bp deletion and type 2 at 5 bp insertion. In all patients, the goal of anagrelide therapy was to control thrombocytopathy (platelet count below 600x10^9/L), with dosage within the range of efficacy and safety recommended in the datasheet. The results were analysed with the statistical software SPSS vs 15.0

Results: 80.5% of the patients were diagnosed with ET, 12.5% of PV, 3.5% of myelofibrosis and 3.3% of unclassifiable MPN. 59% of the patients had a V617F JAK2 mutation, with allelic load higher than 20% in 47.5% of the cases. 28.5% presented mutation in calreticulin; of which 50% were type 1 and 50% type 2. Only one patient had a mutation in MPL (2%), the remaining 6% being classified as “triple negative”. The median daily dose of anagrelide received was 1.5mg. 17.5% of the patients required more than 2mg for an adequate control, half of them being positive for mutations in calreticulin and the other 50% of the muta-
tion V617F JAK2 with allelic load higher than 20%. 26% of the patients received daily dose of 1mg, being 70% positive for the mutation V617FJAK2 with allelic load lower than 20%, although there were no statistically significant differences between the groups according to the mutational profile. 16% of patients dis-
tinued treatment due to toxicity, with the most common adverse effects being mild (headache and palpitations).

Summary/Conclusions: Patients requiring higher doses of anagrelide present mutations in calreticulin or JAK2 V617F allelic load higher than 20% and patients with lower allelic load having greater sensitivity to the drug, with no statistically significant differences. It is possible that the first situation is asso-
ciated with a greater pre-mitotic deregulation in the megakaryocyte where the drug does not interfere whereas the second one could be related to anagrelide interference through the JAK2 pathway in post mitotic maturation although larg-
er exploratory studies are required.

THE DELAYED DIAGNOSIS OF PHILADELPHIA-NEGATIVE MYELOPRO-
LIFERATIVE NEOPLASMS (MPN) IS COMMON AND RESULTS IN A HIGH INCIDENCE OF POTENTIALLY PREVENTABLE THROMBOTIC COMPLI-
CATIONS
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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 mor-
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. Patients with PV had a median time to diagnosis of 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 endovascular 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-
 rhagic events. Earlier recognition of FBC abnormalities consistent with MPN and earlier referral for specialist haematological management would, with earlier intervention, would be expected to prevent many thrombo-haemor-
 rhagic complications and reduce MPN-associated morbidity and mortality.

LONG-TERM AND LOW-DOSE BUSULFAN IS SAFE AND EFFECTIVE IN ELDERLY PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA
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Background: Therapeutic options for elderly patients (pts) with Essential Thrombocythemia (ET) resistant or intolerant to hydroxyurea (HU) are limited. Busulfan (BU) is a possible second-line treatment, but conventional schedule
Background: Thrombosis is one of the most frequent events in Ph(-) myeloproliferative neoplasms and the reasons for that are still under investigation.

Aims: The aim of this study was to find out if there is difference in frequency and type of thrombosis in JAK2 V617F positive patients according to their diagnosis, age, sex and V617F allele burden.

Methods: One hundred and eighty two JAK2 V617F positive patients diagnosed with polycythemia vera (PV) N=63, essential thrombocythemia (ET) N=83, and primary myelofibrosis (PMF) N=36 were included in the study. Patients in each group were additionally divided according to sex, age at diagnosis and first thrombosis. V617F allele burden was quantified in peripheral blood granulocyte DNA by real time PCR established by Larsen et al. Br J Haematol 2007;136:745.

Results: Among 182 patients observed, 66 (36%) experienced thrombosis, with arterial thrombosis being twice more frequent than venous thrombosis in all 3 studied groups. In ET group there was statistically significant difference in sex distribution (proportion of females=0.71), p<0.001. Statistically significant difference in age at diagnosis was observed between ET and PV/PMF patients without thrombosis (p<0.001); the youngest patients were those in ET group. The age at diagnosis of ET patients with thrombosis (65 years, range 23-92) was statistically different compared to ET patients without thrombosis (50 years, range 21-83), p=0.002. Our study showed that V617F allele burden in patients without thrombosis was statistically significantly different between ET (17,2%, range 4,2-55,2) compared to PV (43%, range 1,7-99,9) and PMF (37,1%, range 1,4-90,7), p<0.001. The same statistically significant difference for V617 allele burden was established in patients with thrombosis between ET patients (19%, range 1,4-84,5) and PV and PMF patients (42,5%, range 8,9-97,2 and 48,8%, range 1,6-99,8, respectively), p<0.001.

Summary/Conclusions: Our results confirm that arterial thrombosis is more frequent than venous thrombosis in JAK2 V617F positive patients. Female sex was prevalent only in ET group. The age at diagnosis in all studied groups was similar except for ET patients without thrombosis. There was no difference in the frequency and type of thrombosis among ET, PV and PMF patients with high heterogeneity in V617F allele burden between all studied groups regardless of the occurrence of thrombosis.

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.
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 tetranspan 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 tetranspan 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 tetranspan CD37 as a novel tumor suppressor that directly protects against B cell lymphomagenesis, and provides a strong rationale for blocking the IL-6 pathway in patients with CD37-negative B cell malignancies as therapeutic intervention.

E1355
IDENTIFICATION AND CHARACTERIZATION OF THE LYMPHOMA INITIATING CELL (LIC) POPULATION IN AN ALCCL MOUSE MODEL
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Background: In 60% of anaplastic large cell lymphoma (ALCL) patients a translocation (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 transplant model resembling human ALCL. Therefore we use an inducible Cre/IoxP 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 recipient mice. We observed the formation of CD4 and CD8 double negative T-cell lymphomas. With a latency of 4-5 months, mice developed CD30-positive lymphomas and died from neoplastic T-cell infiltration of lymphatic organs and bone marrow.

Results: Immunophenotypic analysis confirmed T-cell origin of the lymphomas with a heterogeneous combination of all T-cell stages with mainly CD4/CD8- double negative (DN) T-cell subpopulations including all DN T-cell subpopulations as well as hematopoietic stem cells and lymphatic precursors. Staining of the T-cell subpopulations demonstrated high NPM-ALK expression in immature CD4/CD8- double negative T-cells and undifferentiated CD4+CD8+ double positive T-cells with highest expression of proliferation marker Ki67 as well as the activation marker CD25 in the CD4−CD8− T-cells. We selected CD4+CD8− CD4+CD8− double negative lymphoma population further more aberrantly expressing the T-cell receptor alpha/beta chain, which may allow these early T-cells to establish a systemic lymphoma. To further proof this hypothesis and identify the LIC population we performed secondary transplantations with sorted DN T-cells including all DN T-cell subpopulations as well as hematopoietic stem cells and lymphatic precursors. Staining of the T-cell subpopulations demonstrated high NPM-ALK expression in immature CD4/CD8- double negative T-cells and undifferentiated CD4+CD8+ double positive T-cells with highest expression of proliferation marker Ki67 as well as the activation marker CD25. The CD4+CD8− T-cell subset showed the highest expression of NPM-ALK and presented with 100% engraftment, with a more aggressive phenotype as compared to CD4+CD8+ double positive T-cells. Features were compatible with a high-grade lymphomas. IHC analysis confirmed positivity for CD22 staining (Tdt, Bcl6, CD138 and CD4, CD8 negative). Tumors were confirmed to be B220+IgM+, with either Igk-or Ig-lambda-restriction as demonstrated by flow cytometry; and either mono- or bi-tropic population as demonstrated by Southern blotting. Whereas xenografted mice selected from B220+ selected cells obtained from pathological lymph nodes of CD19/Bl/p53- mice and identified 143 SNVs. Non-syonymous 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 lymphoma tumors were implanted s.c. into recipient SCID/Bg mice, and presented with 100% engraftment, with a monomophic lymphomoid infiltration of B220+ and IgM+ cells. B220 positive cells were selected from the s.q. tumor and intravenous injected into recipient SCID/Bg (n: 10) and BL/6 mice (n: 10). Engraftment was demonstrated in all three groups where hepatomegaly and splenomegaly were absent and spleen observed. Infiltration of B220+ cells was documented within bone marrow, liver and spleen. Finally, we found that B220+ selected cells 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 notion that neoplastic DN T-cells incorporate pathways that are present in patients with ABC-DLBCL, thus providing a novel model for studying high-grade B-cell lymphoma driven by BLIMP-1/p53 dual loss-induced C-Myc expression.
ray analyses. Indeed, heatmap analyses revealed wide pattern similarities in the clonal DNA and RNA 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 driving stem-cell-like population originating within the DN3/DN4 lymphoma cell population in a highly relevant NPM-ALK positive CD30-expressing ALCCL mouse model, thereby giving the opportunity to test the eradication of the LIC with established and new therapeutic approaches.

E1356
HSP110 SUSTAINS MYD88-DEPENDENT NFKB SIGNALING IN ACTIVATED B CELL DIFFUSE LARGE B CELL LYMPHOMA
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Background: Diffuse large B cell lymphoma (DLBCL) is an aggressive lymphoproliferative disorder of B lymphocytes accounting for 30% of adult Non Hodgkin Lymphoma (NHL). Among DLBCL, Activated B Cell – DLBCL (ABC-DLBCL) is the most aggressive form and has a poor prognosis. Heat-shock proteins (HSPs) are molecular chaperons highly expressed in cancer cells and implicated in resistance to radio- and chemotherapy. Therefore, HSPs are envisioned as therapeutic targets in many cancers. Among the different HSPs, HSP110 has been recently identified as a pro-survival factor in germline centric-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-DLBC patient samples and several cell lines. SHNAs specific for HSP110 was designed and transcribed through a lentiviral vector designed to infect highly efficiently non-permissive B cell lines. The study was performed with ABC-DLBC patient samples and several cell lines. SHNAs specific for HSP110 was introduced through a lentiviral vector designed to infect highly efficiently non-permissive B cell lines. ShRNA specific for HSP110 was introduced through a lentiviral vector designed to infect highly efficiently non-permissive B cell lines. The study was performed with ABC-DLBC patient samples and several cell lines. SHNAs specific for HSP110 was introduced through a lentiviral vector designed to infect highly efficiently non-permissive B cell lines.

Results: We observed a high HSP110 expression in all ABC-DLBC patient samples, compared to normal reactive lymph nodes by using IHC staining of ABC-DLBC patient tumors. Furthermore, shRNA silencing of HSP110 decreases the survival of several ABC-DLBC cell lines, and downregulates the expression of pro-survival factors such as Bcl2 and Bcl-XL. Sirna silencing of HSP110 abrogates NF-kB silencing, which is the major oncogenic pathway in ABC-DLBC cell lines. In accord with these results, over-expression of HSP110 in DLBCL and non-DLCL 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 DuolinkTM assays, we identified an 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 demonstrated 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-DLBC. 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 NEUTROPENIA THROUGH FAS LIGAND SECRETION
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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare chronic lymphoproliferative disorder characterized by the clonal expansion of CD3+ Large Granular Lymphocytes (LGL). In addition to the most common CD8+ T-LGLL, CD4+ T-LGLL 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 tyr 705 levels were studied by Western blot. FAS ligand mRNA levels were analysed by RT-PCR Assays.

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+/CD8+/dim/neg (CD4+ T-LGLL). All STAT3 mutated (n=38) and almost all neutropenic (38 out of 39) patients belonged to CD8+ T-LGLL leukemia (n=68), while among CD4+ T-LGLL leukemia (n=33) no STAT3 mutated and only one neutropenic patient (1 out of 33, 3%) was found. Among CD8+ T-LGLL, immunophenotypic signature CD16+/CD56- was also associated to the presence of neutropenia and STAT3 mutation (37 out of 41, 90.2%, c2=49.5, p<0.0001 and 37 of 41, 90.2%, c2=49.5, p<0.0001 respectively). Furthermore, by western blot we showed that high STAT3 tyrosine phosphorylation observed in LGL obtained by CD8+ T-LGLL patients belonging to CD16+/CD56- subgroup was significantly higher as compared with other immunophenotypic groups. Provided this relationship between STAT3 mutation/activation and neutropenia, by RT-PCR we analysed Fas ligand expression, showing higher transcription levels in CD16+/CD56- CD8+ T-LGLL patients as compared to the not neutropenic patients belonging to the other immunophenotypes, both CD8+ T-LGLL and CD4+ T-LGLL (7.66±0.87, 2.45±0.22 and 2.35±0.28 arbitrary units, respectively; p<0.01). To confirm this relationship, in patient’s PMBCs treatment with STAT3 inhibitor Static decreased both STAT3 phosphorylation and Fas ligand transcription as compared to the untreated conditions. In addition, IL-6 and IL-15 stimulation (which are known STAT3 activator) increased Fas ligand transcription levels (1.59- and 2.01-fold after IL-6 and IL-15, respectively) which is prevented by concomitant Static treatment.

Summary/Conclusions: Our results provide evidence that STAT3 mutation and activation is mostly restricted to neutropenic CD8+ T-LGLL patients equipped with the CD16+/CD56- signature. The relationship between STAT3 activation and neutropenia FAS ligand related further supports to approach STAT3 inhibition as therapeutic strategy in symptomatic CD8+CD16+/CD56- T-LGLL patients, obtaining the dual results of inducing apoptosis in leukemic LGL together with inhibition to FAS ligand mediated neutropenia.

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.

Figure 1.
Aims: Here, we aim to recapitulate MCL in a mouse model of hematopoietic-specific overexpression of cyclin D2. Next, we want to use this preclinical mouse model to evaluate novel therapeutic strategies for the treatment of MCL.

Methods: To evaluate if cyclin D2 could act as a bona fide oncogene in the pathogenesis of MCL, we developed a 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. Infiltration of the (B)1) into the bone marrow and the lymphoma cells expressing 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 in hematological malignancies. HDAC6 is an atypical member of HDAC family that regulates the acetylation status, and thus the functionality of cytosolic proteins, and has been explored therapeutically for its role in the process of protein degradation. HDAC6 mediates the transport of protein aggregates to the autophagic machinery to diminish their cytotoxicity. Thus, the disruption of the aggresome pathway, similarly to proteasome inhibition, results in a massive accumulation of misfolded protein aggregates and apoptotic cell death. As this inhibition more than 30 months after diagnosis. After ASCT, 41 patients (43%) were randomized in the rituximab maintenance arm and 40 (42%) in the observational arm. Overall, 68 recurrently altered regions were observed in 96% of patients. Deletions were more frequent than amplifications, at 9 vs 3 by patient respectively. HR patients were associated with TP53del (44% vs 14%; p=0.04), CDKN2A del (56% vs 22%; p=0.04), Bp1del (44% vs 15%; p=0.05). Interestingly, we identified in 76% of patients a deletion of a minimal common region of 5.3 Mb located on chromosome 7p22 and including CARD11. This lesion was associated with low MIP (80% vs 12%; p<0.001), and other gains such as 21q21, 10q11 and 6p21 which together define a favorable subgroup (24% of the cohort). These anoma- lies were significantly more associated with LR patients (87% vs 60%; p=.02). None of the patients with CARD11 duplication (n=10) had relapsed despite the presence of TP53 in 2 patients or CDKN2A deletion in 3 patients. This translates into a longer PFS (100%vs70%; p=0.02) (fig.).

Figure 1.
Summary/Conclusions: Our study confirms the worse impact of TP53 and CDKN2A deletion on early relapse in MCL. By contrast, the CARD11 duplication
is associated with an absence of relapse and thus defines a new group of very low risk patients. These findings provide important clues for future therapeutic-driven therapies in MCL.

E1361

CLINICOBIOLICAL FEATURES OF B-CELL NEOPLASMS WITH CDK6 TRANSLOCATIONS: FREQUENT ASSOCIATION WITH MARGINAL-ZONE LYMPHOMA, CONTINGENT OF PROLYMPHOCYTIC 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;11)), leading to juxtaposition of CDK6 gene with IGK, IGH or IGL locus respectively.

Aims: The Groupe Francophone de Cytogenetique Hematicque (GFCH) collected 35 chronic B-cell disorders with CDK6 translocation in order to document the clinicobiological features of this uncommon aberration.

Methods: Clinical and biological data were gathered at diagnosis when available. A cytogenetical review was performed by 3 experts in 27/35 cases. FISH was used to detect IG or TRAD and CDK6 rearrangements, and recurrent abnormalities frequent in SMZL and CLL (trisomy 3, 12, 18, deletions of ATM, TP53, 13q14 and 7q22/7q36 loci), TP53 (exons 4-9), NOTCH2 (exon 34), and IGHV genes were analyzed by Sanger sequencing. Detection of MYD88 L626P was performed by real-time AS PCR.

Figure 1. 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;14)(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%) unclassified small B-cell lymphomas (USBCL) and 5 (14%) chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with Matutes score 4/5 (including the (t(7;14) IGH). Morphopathological review showed a contingent of prolymphocytes (median: 10% of lymphoid cells) in 17/27(63%) cases, including 14/19 MZL and 3/4 USBCL and 0/4 CLL/SLL. The CDK6 translocation was the sole aberration in 9/35(26%). K was complex in 18/35(51%). The most frequent additional abnormalities were: del17p (TP53) (51%), del13q (41%), del8p (23%), trisomy 18 (22%), trisomy 18p (17%) and trisomy 12 (11%). Deletion of 7q and 11q were rare (one case each). TP53 was mutated in 6/22 patients (27%), including 5 with del1p. Overall, 19/29 (66%) tested cases had a TP53 abnormality (del and/or mutated), which was significantly associated with complex karyotype

(p=0.016) and del13q (p=0.042). MYD88 L626P was detected in 2/22 cases. No NOTCH2 mutation was found, IGHV analysis showed a preferential usage of VH4 (8/23, 35%), while VH1 was rare (3/23, 13%, including one VH1-2). Most carried IGHV with some impact of somatic hypermutation (85%). Median follow-up was 28 months [0-192]. The median survival was not reached, only 4/32 (12.5%) died. A treatment was undertook in 15/32(47%) cases, with a median follow-up at first treatment of 13 months. In our series, the CDK6+ MZL cases differed from classical SMZL by frequent prolymphocytic differentiation (14/19, 74%), very low incidence of 7q deletion (1/23, 4%), high frequency of TP53 abnormality (12/23, 52%), absence of NOTCH2 mutation (0/3, 0%), and a different IGHV repertoire with low frequency of VH1-2 (1/11). The CDK6+ USBCL also had frequently a contingent of prolymphocytes (3/4, 75%), and showed a genetic profile similar to the CDK6+ MZL (see figure).

Summary/Conclusions: These results, obtained on the largest series to date, suggest that CDK6 translocation is associated with indolent small B-cell lymphomas, mostly SMZL, with distinctive features. However, CDK6 translocations were rarely observed in other phenotypes. We describe one case involving the T-cell receptor (TCR) locus, which is a rare event in B neoplasms. Finally, it is intriguing that this abnormality involves almost exclusively the IGK locus, and not the other IGI loci, especially IGH which is usually the most frequently rearranged.

E1362

PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE, EXPRESS SPECTROTYPE B-CELL RECEPTORS WITH UNIQUE NONSYNONYMOMATICALLY 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 undefined. Previous studies on small cohorts suggested that DLBCL, LT expresses IGH 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 (Dührren-von Minden, Nature 2012) and non-leg-type ABC-DLBCL (Koning, AACR 2016 & ASH 2016).

Methods: 8 cases of DLBCL, LT were subjected to RNAseq. Additional RNAseq data from 6 healthy volunteers (GEUVADIS project), 10 non leg type DLBCL, and 16 follicular lymphomas were obtained from NCBI publicly available datasets and collaborators. VDJ and rearrangements and IGH constant regions were Sanger sequenced for all cases and two granulocyte controls. Lymphoma-dervised, BCR were tested for autonomous signalling activity in the murine TKO pre-B-cell system (Dührren-von Minden, Nature 2012).

Results: RNAseq analysis demonstrated an IGH isotype in all eight and VJ-kappa in seven of the eight cases of BCL, LT cases. IGHV 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 C region mutations or in the other 32 RNAseq libraries. Constant region mutations were highly specific to DLBCL, LT as compared to other BCL (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 taxomycin.

Summary/Conclusions: Our data identify a clearly stereotyped receptor in DLBCL, LT. In contrast to CLL and ABC-DLBCL, BCR stereotypy was not associated with autonomous BCR signalling activity using a murine IgM backbone. The pathogenic potential of the novel constant region mutations for BCR activity in DLBCL, LT warrants further functional studies.

E1363

LOSS OF NR4A1 ACCELERATES MYC-DRIVEN LYMPHOMAGENESIS ACCOMPANIED BY OVEREXPRESSION OF GENES INVOLVED IN IMMUNOREGULATION

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Background: NR4A1 (Nur77) belongs together with NR4A2 (Nur1) and NR4A3 (NOR-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 RQ-PCR was performed on selected tumor specimens, whereby genes, found to be associated with NR4A1 expression in the publicly available gene expression data set of DLBCLs generated by Lenz et al., were taken. Moreover, the driver function of Nr4a1 in lymphomagenesis at the premalignant stage was investigated by using apoptotic assays and by carrying out transplantations of tumor cells into wt recipients.

Methods: Kaplan Meier analysis was performed for survival and tumor formation in EµMyc Nr4a1-/- (n=154), EµMyc Nr4a1 +/-(n=54) and EµMyc Nr4a1 +/- (n=59), respectively. For RQ-PCR selected tumor specimens from wt and EµMyc mice with (n=14) and without (n=17) Nr4a1 loss were used. For investigation of the role of Nr4a1 at the premalignant stage, mice aged 4 weeks (n=4 per genotype) were sacrificed and AnnexinV staining and cleaved-caspase3 assay were performed on cells isolated from the spleen and bone marrow. In vivo growth of Nr4a1-/- EµMyc tumor cells was induced by tumor cells from the premalignant stage (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 in Nr4a1-/- EµMyc 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-/- EµMyc tumor cells were significantly more sensitive to apoptosis than the Nr4a1-/- EµMyc tumor cell subpopulation isolated at the premalignant stage, whereas apoptosis was significantly diminished in EµMyc Nr4a1-/- mice. RQ-PCR showed that several genes involved in immunoregulation and Nr4a1 target genes were upregulated in EµMyc Nr4a1-/- compared to EµMyc Nr4a1 +/-. Last, tumor formation upon i.v. injection showed that tumors developed faster than tumors derived from mice without Nr4a1 loss (25 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 seems to impact cell death early in B cell development, even ahead of malignant transformation. Additionally, Nr4a1 seems to be involved in driving immune responses towards an anti-inflammation, 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 SPECIES

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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 in vitro cell lines and in vivo murine models have been used to decipher the pathogenesis of CHL. 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 processing can be limited because of poor DNA quality and difficulty to enrich neoplastic cells. Therefore, new enrichment techniques are necessary to enable large scale comprehensive genetic investigations of CHL.

Aims: Our aims were: 1) to develop a technique for HRSC enrichment from the archival formalin-fixed paraffin embedded tissue; 2) to reliably detect genetic aberrations in the genomes of enriched tumor samples and to use this information for development of new prognostic and predictive markers as well as for better understanding of the genetic background of CHL.

Methods: We have developed a new high-throughput method for marker-based enrichment of archival FFPE tissue-derived HRSC nuclei by fluorescence-assisted cell sorting (FACS). Genomic DNA extracted from sorted nuclei was used for identification of mutations in 68 genes that are frequently mutated in lymphomas by targeted high throughput sequencing (HTS). Chromosomal copy number aberrations were investigated by the Agilent SurePrint 180k microarray.

Results: Enzymatically extracted FFPE tissue-derived cell nuclei retain their antigenicity and can be reliably labelled with monoclonal antibodies against nuclear (MUM1, PAX5) and cytoplasmic/cell surface (CD30) markers. A mean neoplastic cell purity of 70% (range 40-95%) was achieved by sorting HRSC cells according to their double expression of MUM1 and CD30 in 11 CHL cases. Using sorted non-malignant cells as a germline control we detected somatic single nucleotide mutations and indels in all investigated samples. Mutations of STAT6, 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 high-quality 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
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 analysis was performed for lineage-specific surface marker expression and identified as B-cells. Malignant B-lymphoid STAT1-deficient cell lines were established and expression levels of typical lymphoid-specific tumor suppressor and promoter genes were assessed by qPCR. In parallel, STAT17-mice were used for RNA-seq analysis to identify the signaling pathways driving disease. RNA-seq data were compared to publicly available RNA-seq data from different haematological 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 masked the development of a B-cell malignancy which can be transferred by CD19+ cells. The malignant B-cells arising in STAT17-mice can be maintained in vitro and display alterations in gene expression that are typically found in human DLBCL such as Irf4, Pdrr1 and p53. RNA-seq analysis revealed features shared with human DLBCL: increased reads a locus of Bim, Meto2, Card11 and Cst7 (PDL1) and decreased expression of Socs-1, Cdkn1a, B2m and Pdrr1. Low levels of STAT1 accumulation combined with low levels of p16INK4A correlated with a reduced life expectancy in DLBCL patients.

Summary/Conclusions: Loss of STAT1 in Balb/C mice provokes a myeloid hyperplasia which masks a B-cell malignancy resembling human DLBCL. DLBCL patients with low levels of STAT1 have a poorer prognosis if they lack the tumor suppressor p16INK4A.

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 from 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 with the COSMIC (Wellcome Trust Sanger Institute), dbSNP and PubMed databases. Exonon 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. JRG1), genes found in invasive carcinoma (e.g. integrin β4 subunit) and genes involved in T helper type 1 and 2 responses (e.g. IL13, IL4, IL6 and PLAG1). Genes involved in cell cycle progression or involved in immune reactions were not shared by all 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 in another patient present at diagnosis 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 in another patient present at diagnosis 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. 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imential conditions we found that a number of the captured genes corresponded to experimentally validated targets of miR-155. Crucially, ontogeny analysis of the PAR-CLIP-captured genes demonstrated an enrichment of genes involved in haematopoietic and/or lymphomagenesis pathways.

Summary/Conclusions: To fully understand the role of a particular miRNA in a specific malignancy, it is essential to identify its target genes in a relevant cellular context. Using a haematopoietic malignancy model of high clinical interest we have developed an optimised method for interrogating the miRNA:mRNA interface (targetome) within a cellular system without the need of ectopically expressed Ago2, keeping physiological levels of the core components of the RISC complex unaffected. Moreover, our optimized protocol allowed us to reduce the number of input cells, therefore opening the exciting possibility of interrogating the targetome of patient primary samples.

E1369

DARATUMUMAB, A NOVEL HUMAN CD38 MONOCLONAL ANTIBODY FOR THE TREATMENT OF B-CELL NON-HODGKIN LYMPHOMA

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: 
- **In vitro**
- **In vivo**

Results: 
- DARA showed significant cytotoxic activity in MCL and FL cell lines in both monotherapy and combination settings.
- In vivo, DARA demonstrated significant antitumor activity in preclinical models.

Summary/Conclusions: DARA represents a promising therapeutic option for the treatment of MCL and FL, showing both monotherapy and combination potential.

E1370

ECTONUCLEOTIDASES CD39/CD73 ARE HIGHLY EXPRESSED ON ATL CELLS AND RESPONSIBLE FOR GENERATING AMP/ADENOSINE

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Background: Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm, linked to the human T-cell lymphotropic virus, HTLV-1. Patients with ATL are often at the risk of opportunistic infections. It might be possible that this immunocompromised state could be induced by the function of ATL cells having similar phenotypes with regulatory T cells (Tregs). However, difficulties of in vitro studies using primary tumor cells have hampered the progress of ATL research, and it is still controversial whether ATL tumor cells have the immunosuppressive characteristics.

Aims: In this study, we analyzed the roles of molecules expressed in ATL cells associated with immunosuppressive functions of Tregs.

Methods: The protocol of this study was approved by the Institutional Review Board of Osaka University Hospital. Peripheral blood mononuclear cells (PBMCs) were collected from 8 asymptomatic HTLV-1 carriers and 20 ATL patients (3 with smoldering type, 5 with chronic type, and 12 with acute type) after getting informed consent. PBMCs from 3 ATL patients were separated into CD4+CD7-CADM1+ ATL cells and adjacent CD4+ CD7+CADM1 normal T cells using Fluorescence-activated Cell Sorter (FACS), and total RNA sequencing experiments were conducted. And we also examined the expression patterns of CD39 and CD73 in vitro and in vivo carriers or each type of ATL patients.

Results: We compared whole transcriptome of ATL cells and normal CD4+ cells. Bioinformative analyses showed that many genes associated with immunosuppressive functions of Tregs were elevated or downregulated in ATL cells. Among these genes we focused on CD39 and CD73, because recently it has been reported that extracellular adenosine, which is catalyzed by CD39, expressed in human Tregs, and CD73, expressed in murine and human Tregs, has strong anti-inflammatory function and plays major role in Treg-mediated immunosuppression. Therefore, we investigated the expression of CD39 and CD73 in ATL cell lines and primary tumor cells. We found that all of 4 ATL cell lines expressed CD39, but not CD73 just as human effector Tregs. In contrast, the expression patterns of CD39 in 20 ATL patients were various (Table) and interestingly, some ATL tumor cells express CD73. Also in asymptomatic carriers, we could detect CD39 and/or CD73 positive on CD7+CADM1+ abnormal fraction of CD4+ cells. CD26, expressed in human naive but not in effector Tregs, was negative in all cell lines and primary cells except for abnormal cells in one smoldering patient. Next, the role of CD39 and/or CD73 in ATL cells was assessed. Extracellular ATP is converted to AMP by CD39. As expected, CD39+ ATL cells converted significantly more ATP than CD39+ ATL cells, which were comparable with normal effector Tregs. Conversely, mass spectrometry analysis of AMP/adenosine concentration indicated the activity of CD73 mediated AMP hydrolysis was very slow; less than 10% of 1mM ATP was converted to adenosine by CD73+ ATL cells, indicating that the aberrant expression of CD73 could not efficiently increase adenosine synthesis.

Summary/Conclusions: In this study, we showed that about two thirds of ATL samples were CD39+CD26+ just as effector Tregs and have comparable level of ATPase activity as Tregs, which are expected to play some immunosuppressive function in ATL patients. Recently it is also reported that in exhausted CD8+ T cells in cancer patients, CD39 is co-expressed with PD-1. CD39 expression in ATL cells may also have some roles in immunosuppression and thus in the escape from anti-tumor immunity.
and proliferation of several B cell malignancies. BTK is a key regulator of this pathway. In a preliminary clinical study, the selective BTK inhibitor STRO-001 showed therapeutic activity in relapsed/refractory DLBCL of the Activated B-cell phenotype (ABC-DLBC) (Walter et al Blood 127 pp411-419, 2016). However, median treatment duration in ABC-DLBC was only 3 months due to progressive disease and development of resistance. Two acquired resistant mutations C481S and R665W have been reported as dominant resistant 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 insensitive ABC-DLBC cell line TMD8 was cloned ONO/GS-4059 and Ibritumomab resistant TMD8 cell lines (TMD8RO and TMD8RI) were used for this study. TMD8RO has PLC2y665W whilst TMD8RI lacks both BTK C481S and PLC2y665W. Cell viability and apoptosis after compound treatment were assessed using Cell titer Glo assay and Annexin V/PI staining. Expression of ONO/GS-4059 in intracellular glycosylation status and expression of immunoreceptor were assessed by immunoblot and Flow cytometry. The mutational status of BTK and PLC2y in TMD8 was determined by Sanger sequencing.

**Results:** ONO/GS-4059 induced apoptosis in TMD8 at nanomolar concentrations in >70% of cells. Although ONO/GS-4059 induced rapid reduction in ERK and AKT activation, activation of ERK and AKT rebounded within 24 hours in surviving cells. Interestingly, surface immunoglobulin M (sigM) expression was increased more than three times in these cells leading to subsequent activation of SYK. The specific SYK inhibitor GS-9973 combined with ONO/GS-4059 inhibited the downstream ERK and AKT reactivation and induced synergistic apoptosis in TMD8. On the other hand, SYK hyper-activation as determined by phosphorylation of SYK and its downstream target BLNK was also observed in the two BTK inhibitor resistant cell lines. Additionally, expression of CDS and CD22, which negatively regulate BTK, was decreased in these cells. The combination of ONO/GS-4059 and GS-9973 restored sensitivity to ONO/GS-4059 and induced synergistic apoptosis in both resistance cell lines.

**Summary/Conclusions:** These data show that SYK is highly activated through increased sigM expression and/or downregulated CDS and CD22 following BTK inhibitor treatment. 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.

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**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 readily 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 antibody-drug conjugate (ADC) containing anti-CD74 aglycosylated human IgG1 antibody (SP7219) conjugated to a non-cleavable dibenzocyclooctyne (DBCO)-maysitindoside 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-to-antibody ratio (DAR) of 2. Due to its limited cell permeability, the major catabolite released by STRO-001 has 100X lower cell killing activity on CD74 positive and negative cells compared to a reference cytotoxic maytansine. Since conjugation sites were selected based on highest stability both in vitro and in vivo, thereby limiting loss of drug moiety from STRO-001 in circulation, this novel ADC has potential for improved safety and activity.

**Aims:** The aim of this study was to investigate the therapeutic potential of STRO-001 in non-Hodgkin’s lymphoma (NHL) cell lines and xenografts. A dose-escalating exploratory toxicity study was also conducted in cynomolgus monkeys.

**Materials and Methods:** Biotinylated SP7219 was used for immunochemistry (IHC). DBCO-Alexa647-conjugated SP7219 and flow cytometry were used for detection and quantitation of CD74 expression on NHL cell lines and B-cells from normal human donors. STRO-001 was used to determine the EC50 and percent span of killing in NHL cell lines. The anti-tumor activity of STRO-001 in SCID mice bearing human xenografts was measured as tumor inhibition in the xenografts and compared to control xenografts. We also tested the effect of STRO-001 on TMD8 cell lines, which were derived from a human diffuse large B-cell lymphoma (DLBCL), 2 activated B-cell lymphoma (MCL) cell lines with EC50 values ranging from 0.17-13 nM. STRO-001 has only modest effects on naïve B-cells, but exhibits more potent cell killing in activated human B-cells that have upregulated CD74 expression (similar CD74 expression as SU-DHL-6 cell line). CD74 cell surface expression is required for STRO-001 cytotoxic activity but expression level, as measured by antibody-binding capacity, does not correlate with in vitro potency (R2=0.4154). STRO-001 exhibits dose-dependent tumor growth inhibition in rituximab-resistant SU-DHL-6 xenografts starting at 2.5mg/kg weekly x 3 doses. The standard of care combination of bendamustine/ rituximab (BR) + STRO-001 further improves tumor suppression in SU-DHL-6 xenografts compared to vehicle (p<0.001) or BR alone (p=0.02). Studies with a MCL xenograft model, Jeko-1, demonstrate potent anti-tumor activity compared to vehicle (p<0.0001) starting at a single STRO-001 dose of 3mg/kg, with a single 10mg/kg dose resulting in tumor regression for up to 64 days post treatment. STRO-001 treatment 14 days post tumor inoculation was used to evaluate disease progression in the xenografts, as STRO-001 on intracellular glycosylation status and activity in NHL cell lines and anti-tumor activity in NHL xenograft models, including prolonged survival in the disseminated Mino MCL model. STRO-001 depletes B cells in a dose-dependent manner. Clinical studies of this novel ADC for treatment of B-cell malignancies are under development.

**Summary/Conclusions:** STRO-001 demonstrates potent in vitro cytotoxicity in NHL cell lines and anti-tumor activity in NHL xenograft models, including prolonged survival in the disseminated Mino MCL model. STRO-001 depletes B cells in a dose-dependent manner. Clinical studies of this novel ADC for treatment of B-cell malignancies are under development.
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 optimization of the PCR and Southern blot sensitivity, whereas the levels of BCL-2 targets less prone to somatic mutations, such as IGL, should therefore be evaluated. Conversely, 21 samples were PCR+ but FCM-. Absence of FCM detection might have resulted from the presence of very large lymphomatous cells outside the scope of analysis. Also, rapid cell death is an issue with FCM (preventing optimal blocking), and a low tumor cell content might 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 B-cell malignancies. It seems premature to make clinical decisions based on a single technology. Both methods, which suffer limitations that need to be acknowledged, are complementary and should be performed at diagnosis. Specific limitations of each of them should be taken in consideration for follow-up studies.

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**E1375**

**THE SYK INHIBITOR R406 DRAMATICALLY INCREASES THE SENSITIVITY OF GCB AND ABC DLBCL CELL LINES TO THE B-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 (Davies MS et al, J Clin Oncol. 2017).

**Aims:** Investigate whether the SYK inhibitor R406 can increase sensitivity of DLBCL cells in vitro to venetoclax.

**Methods:** The following cell lines were used: Ly1, Ly4, 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/PI staining and flow cytometry analysis. Expression of BCL-2 family members was determined by immunoblotting or qR-PCR analysis.

**Results:** In a recent study, we showed that MCL-1 increases the resistance of anti-IgM stimulated CLL cells to venetoclax, and that SYK inhibitors can effectively overcome this resistance by blocking B cell receptor (BCR)-mediated MCL-1 upregulation (Bojarczuk K et al. Blood. 2016). Since constitutive activation of the BCR pathway has been described in both ABC and GCB DLBCL (Davies RE et al, Nature 2010; Chen L et al, Cancer Cell. 2013), we investigated whether treatment with the SYK inhibitor R406 can sensitize DLBCL cells to venetoclax. Single-agent venetoclax had only modest activity against most DLBCL cell lines at concentrations ranging up to 0.25 μM (Figure 1). Substantial apoptosis induction (>20%) was observed in only 2 GCB (Ly1 and Ly18) and 2 ABC (U2932 and Ly10) cell lines. R406 as single agent had almost no effect on tumor cell viability, with only one cell line showing >20% apoptosis induction (HBL1). However, addition of R406 to venetoclax resulted in a dramatic increase in the percentage of apoptotic cells in six of the investigated cell lines (Ly18, DHL4, U2932, Ly10, HBL1 and TMD8). A synergistic effect was also observed with Ly1 using a lower concentration of venetoclax, whereas no effect or only minimal differences in cell viability were observed in the remaining cell lines (Ly4, Ly7, Toledo, BJAB, DHL2 and Ly3). Among these, only Toledo expressed similar levels of venetoclax sensitivity in the absence of SYK inhibition, whereas in the other cell lines the effect was less pronounced or undetectable. To understand the mechanisms how R406 increases the sensitivity of DLBCL cells to venetoclax, we evaluated changes in the expression of MCL-1 and other antiapoptotic BCL-2 family proteins that have been associated with venetoclax resistance. Five of the seven R406 + venetoclax sensitive cell lines (Ly1, DHL4, U2932, HBL1 and TMD8) showed a 20-45% reduction in MCL-1 levels following 24 hours culture with 2μM R406, whereas no changes were observed in Ly18 and Ly10. However, a substantial reduction in A1 levels was observed in Ly18 and U2932 cells, whereas no substantial changes in A1 and BCL-XL expression were detected in any of the other investigated cell lines. Finally, we also investigated the effects of R406 on expression of HRK, which is a propapoptotic BCL-2 family member that was recently shown to be induced by SYK inhibition in a subset of GCB DLBCLs (Chen L et al, Cancer Cell. 2013). A substantial increase in HRK expression (140-640%) was observed in 5 of the 7 R406 + venetoclax sensitive cell lines (Ly1, Ly18, DHL4, U2932 and TMD8).

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**E1376**

**VB EXPRESSION ASSESSMENT AND CLONALITY DETECTION IN T-CELL PROLYMPHOCYTIC LEUKEMIA (T-PLL) BY FLOW CYTOMETRY (FCM) AND NEXT GENERATION SEQUENCING (NGS): A COMPARISON OF BOTH METHODS**

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**Background:** VB repertoire analysis can distinguish monoclonal from polyclonal (reactive) T-cell proliferations. The molecular quantification of clonal T-cell receptor (TR) gene rearrangements can also be used to record minimal residual disease (MRD) in T-cell malignancies. TR clonality can either be assessed by FCM employing VB antibody panels covering ~70% of the normal human TR VB repertoire or by molecular techniques like NGS with primers that amplify virtually all possible VB-JB rearrangements. T-PLL is the most common (primary non-Hodgkin) T-cell leukemia. Clonal TR gene rearrangements are detected in virtually all T-PLL by FCM or PCR from peripheral blood (PB) or bone marrow samples.

**Aims:** To compare the results of parallel TRB-based clonality analyses by FCM and NGS in T-PLL.

**Methods:** We investigated diagnostic PB leukocytes of 73 T-PLL patients with median lymphocytes at 66% (range 13-93; harvesting T-cells at 97% (55-100)). FCM of surface (not intracellular) VB expression was assessed by the IOTest Beta Mark kit (Beckman Coulter). Libraries for NGS were prepared using 100ng of DNA via a 2-step PCR and sequenced on the Illumina MiSeq (2x250bp, v2) with a median coverage of 17,908 reads (range 1,125–41,193/sample). In the first PCR TRB rearrangements were amplified using TRB BIOMED-2 V- and J-region primers (van Dongen et al, Leukemia 2003). In the second PCR step, sequencing adaptors and sample-specific barcodes were added. Annotation of TR V, (D)- and J-regions of TRB sequences was done using ARResT/Interrogate (Bystry et al, Bioinformatics 2016).

**Results:** In all samples one or two dominant clonal TRB rearrangements were detected by NGS and represented in median by 83% of reads (range 15-90%). In 36/73 (49%) of these cases, also FCM demonstrated clonality. Interestingly, in 8/36 (22%) of cases the dominant VB by FCM differed from the molecular clonotype. In 5 of these cases the discrepancy was most likely accountable to a non-functional TRB clone detected by NGS corresponding to a bi-allelic TRB rearrangement with the second non-functional allele being preferentially identified by NGS. In 37/73 (51%) of cases no reaction with one of the VB antibodies was seen. In 16 (43%) of these cases this could be attributed to expression of a TRB rearrangement for which the appropriate VB antibody was not present in the FCM panel. In another 12 (33%) of these cases a non-productive TRB rearrangement represented the dominant NGS clonotype. However, in further 9 cases (24%), the functional TRB clonotype (TRBV 5-6, 6-5, 25-1, 18, 20-1, 27) was not detected by FCM due to theoretical coverage. Of note, overall 10/73 T-PLL (14%) lacked surface TRβ chain expression.

**Summary/Conclusions:** T-cell clonality is detected by TRB NGS in all T-PLL, whereas FCM-based VB repertoire analysis identifies a dominant single VB.
domain expression in only 49%. A substantial proportion of such failures is due to clonal deletion, which can be explained by the loss of 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 laborious 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.

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 (BTKi) have unexpectedly shown significant clinical effect; however despite this success, approximately one third of MCL patients have primary resistance to the drug, and patients who initially respond to treatment frequently acquire secondary resistance and aggressive relapse of the disease. Understanding how BTKi-resistance or sensi-
tivity is mediated can identify new targets for therapy or predictive biomarkers of response. Using an in vitro model system we have identified the transcription factor IRF4 as a sensitive indicator for BTKi response in MCL cell lines and pri-
mary cells.

Aims: To identify molecules or pathways responsible for resistance to BTKi 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 BTKi sensitive REC-1 cell line was contin-
uously treated with BTKi to generate an acquired resistance model. Cultures were treated with BTKi drugs: ibrutinib or acalabrutinib in the presence or absence of B-cel receptor or CD40L stimulation, and their sensitivity or resist-
tance 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 phos-
phorylation analysis (immunoblotting) and by mRNA expression (RT PCR).

Following initial experiments the studies focussed on IRF4.

Results: Each MCL cell line showed basal phosphorylation of BTK (Y223) and its downstream effector molecule ERK1/2 (T204/187); in each case phospho-
rylation was prevented by BTKi. Of the cell lines tested however, only REC-1 cells showed growth inhibition by BTKi (ibrutinib and acalabrutinib), demon-
strating both dose-dependent apoptosis (p<0.01) and inhibition of proliferation. Further investigation showed that only the BTKi-sensitive REC-1 cell line down-
regulated IRF4 in response to BTKi; this downregulation was an early and spec-
ific response (mRNA downregulated after 4 hours, and protein expression after 8 hours). Furthermore in REC-1 cells with acquired partial resistance to BTKi, the downregulation of IRF4 was significantly less than in the parental cell line. Finally in vitro co-culture of REC-1 cells with CD40L prevented IRF4 downregulation, demonstrated the requirement of CD40L for IRF4 downregulation.

Experiments with primary MCL cells reinforced these findings: in vitro CD40L induced proliferation, survival, prevented BTKi-induced IRF4 downregulation and protected the cells from BTKi-induced death. These findings were confirmed using ex vivo samples from treated patients (n=7) analysed before and during BTKi treatment. IRF4 was downregulated in 6 samples from patients shown to be clinically responding to BTKi and was not downregulated in 1 refractory case.

Summary/Conclusions: CD40L encountered in the cellular microenvironment supports the proliferation and survival of MCL cells, and protects them from the effects of BTKi inhibition. This study has identified that BTKi induces down-
regulation of IRF4 in sensitive but not resistant MCL cells, and that downreg-
ulation is opposed by CD40L. This suggests that the expression of IRF4 fol-
lowing treatment with BTKi might be a biomarker for BTKi-sensitivity in MCL, and that proteins modulated by IRF4 may play an important role in MCL treatment response.

E1379

LIQUID BIOPSY: DECIPHERING A SIGNATURE OF CIRCULATING MICRORNAS AS NOVEL NON-INVASIVE BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA


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Background: While MYC-driven non-Hodgkin lymphomas have aggressive clinical behavior and respond poorly to treatment. However, MYC-dependent lymphogenesis 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 lymphogenesis.

Aims: The aim of this study is to define the impact of and the mechanism by which TPL2 kinase affects MYC-induced lymphogenesis.

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 pro-
genitor cells under the control of the IgH chain enhancer. Mouse pre-B lymphocytes were isolated from bone marrow by flow cytometric cell sorting. Differ-
entiation status of lymphomas was analysed by flow cytometry using B220, IgM and IgD antibodies. The TPL2 RNA and protein expression levels were measured by qPCR and Western blot analysis, respectively. The extent of apop-
tosis 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 lympho-
phocytes of healthy (premalignant) Eμ-myc mice express TPL2 in comparable levels to their WT counterparts, suggesting that the reduction of TPL2 expres-
sion 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 lymphogenesis. 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 abla-
tion of TPL2 in the Eμ-myc mouse model accelerates MYC-induced lymphoma-
genesis 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-99a/miR-125b cluster) and by previously published studies (miR-22, miR-18a and miR-20a) was evaluated by qRT-PCR in cyclin D1-overexpressing DLBCL cell lines. RNA samples collected at diagnosis of the first 18 patients enrolled into the study.

Results: Our results showed that the expression level of serum miR-22 as well as let-7c-miR-99a/miR-125b cluster was significantly higher at diagnosis, in patients unresponsive to R-CHOP treatment when compared with responsive patients. On the contrary, miR-18 and miR-20 levels appeared to be not significantly associated to treatment response. In addition, a global expression profile of circulating miRNAs was evaluated in serum samples derived from a smaller cohort of patients (n=4) after first-line chemo-immunotherapy. Interestingly, we found a striking difference in miRNA modulation upon treatment between responsive and unresponsive patients. In particular, we found 31 miRNAs significantly modulated after R-CHOP in the group of responsive patients, including miR-22. In contrast, this miRNA subset did not show remarkable expression changes in unresponsive patients. Moreover, we performed a study interrogating The Cancer Genome Atlas (TCGA) database about miRNA expression levels in samples of DLBCL patients. We found that the only available data are relative to the miRNA expression levels in tumor tissue samples of 47 out of 58 DLBCL patients. Kaplan Meier method and log-rank test revealed a signature of 13 miRNAs with potential prognostic value. Among these we found that miR-22, also emerged as modulated in our genome-wide analysis, was linked to risk of disease recurrence.

Summary/Conclusions: These preliminary data suggest that the serum miR-22 as well as miR-99a/let-7c-miR-125b miRNA cluster are of potential interest as non–invasive biomarkers to predict therapeutic response in DLBCL patients. Ongoing experiments in a wider cohort of patients are aimed to confirm these results and unveil potential miRNA signature with predictive value.

E1380
INTRACELLULAR CALCIUM AND METABOLISM HAVE CRITICAL ROLES IN DETERMINING ANTI-CD20 ANTIBODY EFFICACY IN DLBCL

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Background: Since the discovery and utilisation of the Type-I anti-CD20 antibody Rituximab, many have tried to enhance the efficacy of anti-CD20 antibodies in order to improve first-line treatment of B cell malignancies, leading to the development of anti-CD20 and anti-CD20-like antibodies. However, the biological role of CD20 and the mechanism of anti-CD20 antibody action remains unclear. However, CD20 has been shown to be involved in the store operated calcium (Ca2+) system. This complex has the ability to facilitate mitochondrial membrane permeabilisation, resulting in reduced mitochondrial function. Basal oxidative phosphorylation (OxPhos), ATP production, and maximal and spare respiratory capacity of cells can be calculated as a measure of mitochondrial function.

Aims: i) Assess and compare intracellular calcium concentration following treatment with anti-CD20 antibodies ii) Evaluate mitochondrial function of cells following treatment with anti-CD20 antibodies iii) Assess whether cytotoxicity of Type-I and Type-II anti-CD20 mAbs can be enhanced by exploiting cellular metabolism

Methods: We established a panel of four DLBCL cell lines (Karpas422, Pfeiffer, OCI-LY7 and SUDHL4). Following a 24-hour treatment with one of four anti-CD20 antibodies (Rituximab) and three Type-II anti-CD20 antibodies (BH2, Obinotuzumab and Tositumomab), intracellular calcium concentration was quantified and visualised using imaging flow cytometry. Next, we used the XF Seahorse Mito Stress Test to reveal bioenergetic profiles of the cell lines following a 24-hour treatment with the same antibodies. We used Metformin (5 mM) as an OxPhos inhibitor and then characterised the bioenergetic profile of our panel of cell lines again, this time to assess how combining each anti-CD20 antibody with an OxPhos inhibitor affected mitochondrial function. Metformin was also used to reduce the mitochondrial membrane potential (MMP) across our panel of cell lines. We confirmed MMP reduction by staining cells with JC-1, a chameleon dye used as an indicator of MMP and analysed samples using flow cytometry. Under the same conditions, we conducted clonogenic survival assays to see whether cytotoxicity of anti-CD20 antibodies could be enhanced by manipulating metabolism.

Results: Intracellular calcium concentration was decreased across our panel of cell lines following a 24-hour treatment with all Type-II anti-CD20 antibodies in our panel. This decrease was not observed following treatment with the Type-I anti-CD20 antibody Rituximab. Treatment with anti-CD20 antibodies resulted in a significant increase in the maximal respiratory capacity of our panel of cell lines; cells were able to produce more ATP in response to oxidative stress. Conversely, pharmacological inhibition of OxPhos impaired mitochondrial function, causing a significant reduction in basal OxPhos and in maximal respiratory capacity. Under this condition, cells were unable to increase ATP production in response to oxidative stress. We also show that treatment combining Metformin with either Type-I or Type-II anti-CD20 antibodies prevents the increase in maximal respiratory capacity observed with anti-CD20 antibody treatment alone. When analysing the clonogenic survival of cell lines, we have found that only the cytotoxicity of Type-II anti-CD20 antibodies is enhanced by simultaneously treating cell lines with Metformin.

Summary/Conclusions: Our data show for the first time that when cells are treated with 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 decreases the cells from being sensitive to chemical inhibitors of OxPhos and Metformin. 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.

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 hematological 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 lymphoid malignancies.

Methods: Chromatin immunoprecipitation (ChIP) followed sequencing was performed in four established MCL cell lines. RNA-Sequentializing (RNA-Seq) and information from histone ChIP-Seq were correlated with genomic intervals displaying cyclin D1 binding. Transcriptional downregulation was studied through cytotmetric RNA total quantification in lymphoblastic cyclin D1-overexpressing models and RNA Pol II ChIP-Seq.

Results: Endogenous cyclin D1 showed widespread binding to active promoters. Its 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 CLASSESS CLASSICAL MCDONALD 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–Stemberg 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 complete 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 nodes 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 risk of relapse, comparing to those without 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 80% for double negative PD-L1+/IDO-patients vs 20% for double positive PD-L1+/IDO+ patients (p=0.008). IL-13 was expressed at various levels depending on the stage of cHL with the highest expression levels in advanced stages. A trend for a higher risk of relapse was observed for HL patients with increasing level of IL-13, (p=0.23). TGF-β expression was checked in 15 cHL patient samples, with 78% analyzed by flow cytometry in order to assess complement-dependent cytotoxicity (CDC) on HL cells. Multivariate analysis showed that TGFβ expression is a significant increase EFS in cHL patients with HRs of 6.7 [95% (CI) 1.3-2.1, p=0.04].

Summary/Conclusions: Our results clearly indicate that HDAC6 inhibition can be used as an effective strategy to be associated with the therapy with anti-PD-L1 mAbs. This strategy seems to be highly promising in cHL patients, known to express low CD20 levels. Moreover, we performed cytotoxic assays using flow cytometry in order to assess complement-dependent cytotoxicity (CDC) as well as apoptosis. We used HDAC6-specific chemical inhibitors (tubacin, trichostatin A and clinically tested ricolinostat) as well as HDAC6 shRNA assay. We also performed animal studies using SCID mice injected with Burkitt CD20+ lymphoma cell line s.c. and treated with ricolinostat i.p. We used both the pharmacological (i.p. administration of ricolinostat) and genetic (cells stably transduced with HDAC6 shRNA) approach.

Results: The results of our studies demonstrate that HDAC6 inhibition significantly increases CD20 level and sensitizes tumor cells to rituximab- and ofatumumab-induced CDC, as well as to direct cytotoxicity of obinutuzumab. In in vivo settings HDAC6 inhibition potentiated the efficacy of rituximab by significantly reducing tumor size and prolonging the survival of the mice.

Summary/Conclusions: Our results clearly indicate that HDAC6 inhibition sensitizes tumor B-cells to anti-CD20 immunotherapy. Therefore, we propose HDAC6 inhibition with specific inhibitors as an effective strategy to be associated with the therapy with anti-PD-L1 mAbs. This strategy seems to be highly promising in cHL patients, often expressing very low CD20 level and do not benefit from immunotherapy.

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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) and as indolent mononuclear lymphoproliferations. Refractory coeliac disease type II (RCDII) is one of the indolent clonal 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 CD38 and the presence of a clonal TCR rearrangement. Lymphocytes from RCDII are dependent for survival on IL-15, which promotes 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 not expressed in the indolent T-LPD (n=15). The NKp46 expression was also associated with a poor prognosis in GI T-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.

E1387

HIGH EXPRESSION LEVELS OF MIR23A CLUSTER IN DLBCL ANTAGONIZE INDUCTION OF APOPTOSIS

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Background: The microRNA cluster MIR23A, which encodes for the mature microRNAs miR-23a, miR-27a and miR-24, was shown to be deregulated in many different malignancies including subtypes of B cell non-Hodgkin lymphoma (B-NHL). Furthermore, high expression of miR-23a was correlated with poor overall survival in diffuse large B-cell lymphoma (DLBCL) patients (Wang et al., Med Oncol. 2014) indicating that miR-23a might act as an onco-miR (tumor promoting microRNA) in this entity. However, both targets and function of the MIR23A cluster in B-NHL remain unknown.

Aims: This study aims to elucidate the role of the MIR23A cluster as a potential onco-miR in DLBCL by identification of the lymphoma-specific targetomes of miR-23a and miR-27a and subsequent analyses of associated functions.

Methods: We identified novel cell line U-2932 K1, which has a low basal expression level of MIR23A cluster, was used for the lentiviral-based generation of clones overexpressing miR-23a, miR-27a, or a scrambled control. Differentially expressed genes (DEG, fold-change >2, p-value <0.05) between samples were determined by miRNA sequencing (RNA-Seq). miR-23a and miR-27a targetomes were identified by immunoprecipitation of AGO2-bound miRNA (AGO2-RIP) followed by RNA-Seq. MicroRNA targets had to be enriched >2-fold with a p-value <0.05. Validations were performed by qPCR and immunoblotting.

Gene set enrichment analyses (GSEA) and GO-term analyses were applied on identified targetomes and DEG to predict microRNA associated functions. Apoptosis was assessed by Annexin-V staining followed by FACS analyses as well as in immunoblots.

Results: Overexpression of miR-23a and miR-27a, respectively, in a DLBCL model cell line resulted in global alterations of gene expression (so-called indirect targets) with a substantial overlap of 104 of DEG affected by both miRNAs. Using AGO2-RIP, 26 novel direct targets of miR-23a, and 20 novel direct targets of miR-27a were identified. GSEA and GO-term analyses of direct and indirect targets indicated that the MIR23A cluster might regulate processes in apoptosis. Moreover, BBC3 which encodes the pro-apoptotic protein PUMA was one of the identified direct targets of miR-23a. As expected, induced apoptosis was reduced in MIR23A overexpressing DLBCL cells failed to induce PUMA on protein-level. Importantly, functional analyses confirmed that miR-23a overexpression reduces and high levels of miR-27a significantly attenuate the ability of DLBCL cells to undergo apoptosis in response to DNA damage.

Summary/Conclusions: We demonstrate that high levels of miR-23a and miR-27a downregulate induction of apoptosis in a lentiviral-based cell line. This might be one possible explanation why DLBCL patients with high miR-23a expression levels have a worse overall survival rate than patients with low levels. Thus, further studies should address the suitability of the MIR23A cluster as biomarker and potential target in DLBCL.
hours and produced 8.7 – 9.3 X 10^3 ng/ml of IgM. PCs isolated from BCWM.1 increased to 130% and produced 2.5 – 2.8 X 10^3 ng/ml of IgM. LPLs from both cells line proliferated in culture (~ 130 – 140% in MWCL-1 and ~170 – 200% in BCWM.1 at 72 hours), gave rise to the more differentiated PCs (7.5 – 9.0% PCs at 72 hours in MWCL-1 and 1.2 – 1.4% PCs in BCWM.1), and secreted smaller amounts of IgM than PCs (3.5 – 5.0 X 10^3 ng/ml in MWCL-1 and 0.3 – 0.7 X 10^3 ng/ml in BCWM.1).

Summary/Conclusions: Our analysis of the 2 WM cell lines provides evidence to support the common hypothesis that malignant PCs arise from the clonal malignant LPL population, and are primarily responsible for IgM secretion in WM.

E1389
LMP-1 MEDIATED UPREGULATION OF IL-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 NKTLCL 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 NKTCL.

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. LMP-1-harboring lentiviral vectors were transfected into NK-92 cells to examine the correlation between LMP-1 and IL-2Rα expression. Proteins in the downstream pathways of LMP-1 signaling were measured in NK-92 cells, at both protein and mRNA levels. Expression of IL-2Rα was remark-ably upregulated in NK-92 cells transfected with LMP-1-harboring lentiviral vectors compared with those transfected with negative control vectors. Proteins in the MAPK/NF-κB pathway were upregulated in LMP-1-expressing NK-92 cells compared with the negative control. Selective inhibitors of those proteins induced the MAPK/NF-κB pathway were upregulated in LMP-1-expressing NK-92 cells compared with those transfected with negative control vectors. Proteins in the downstream pathways of LMP-1 signaling were measured in NK-92 cells, at both protein and mRNA levels. Expression of IL-2Rα was remark-ably upregulated in NK-92 cells transfected with LMP-1-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, D, 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 NKTCL by LMP-1-mediated activation of MAPK/NF-κB pathway. IL-2Rα can promote NKT-CL cell proliferation partially through regulation of cell cycles 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 NKTCL.

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: PD1 binding to its ligand PDL1 inhibits TCR/BCR signaling; impairs activation; and effector functions of T- and B-cells; induces a state of T-cell exhaustion; and ultimately provokes tolerance towards cancers. PD1 is expressed on Hodgkin lymphoma (HL) and B-cell non-HLTTs. The TME may play an essential role in maintaining PD1-induced immune exhaustion. LEN is an oral immunomodulator (IMiD) with direct antineoplastic activity and immune checkpoint activation. This study aimed to evaluate the role of the TME in supporting the PD1 axis; and 3) to determine whether LEN influences PD1 or cognate ligand expression.

Aims: 1) To better characterize the PD1, PD1L and the lesser-known PDL2, phenotype in peripheral neoplastic CD19+ lymphocytes and T-cell subsets in patients with low-grade B-cell lymphoma; 2)to evaluate the role of the TME in supporting the PD1 axis; and 3) to determine whether LEN influences PD1 or cognate ligand expression.

Methods: Samples obtained from patients attending participating Hematology Units were used to determine PD1, PDL1, PDL2 phenotype (%SEM) by Flow-cytometry (FC). Autologous activated T-cells (AAT) were obtained by in vitro co-culture of patient T-cells with anti-CD3/CD28 beads, rIL2 and with PBMCs. Cultures were monitored daily until sizeable clumping was observed and tested for PD1 and ligand expression. In selected experiments LEN(provided by Cel-gene) was added to cell cultures. Results: Twelve cases of lymphoma were evaluated for PD1, PDL1 and PDL2 expression on malignant B- and T-cells by FC. The expression of PD1 and PDL2 was similarly expressed, while PDL1 was almost undetectable on B-cells. Levels of PD1 expression on CD3+ cells were variable across samples, however they were significantly higher than those expressed on malignant B-cells. Significantly higher levels of PD1 expression and very low levels of ligands were detected in both CD4+ and CD8+ cells.CD4+ and CD8+ cells showed consistent formation of B/T-cell clusters. Higher numbers of CD19+CD56+PD1L+ cells were detected than PDL1+ cells compared to baseline cells. PD1 expression also significantly increased in AAT co-culture on B-cells. PD1 expression
on CD3+ cells was unaffected by AAT, although the expression of both ligands remained 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 (0.5–1µM) 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, PD-L2 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 PD1L expression by LEN in CD8+ cells.

**Summary/Conclusions:** Our data provide support for the potential involvement of the PD1-axis in lymphoma patients. Interestingly, LEN further induces the expression of PD1 in CD8+ and CD4+ cells and may contribute to reactivating PD1 signaling under treatment. The PD1 pathway may be potentially targeted to overcome both the intrinsic and LEN-induced exhaustion phenotype.

**E1391**

**IDENTIFICATION AND DIAGNOSTIC APPLICATION OF GENOMIC NPM-ALK FUSION SEQUENCES IN ANAPLASTIC LARGE CELL LYMPHOMAS**

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**Background:** ALK positive anaplastic large-cell lymphoma (ALCL) account for 10-15% of pediatric Non-Hodgkin lymphomas. Most of these patients carry the chromosomal translocation t(2;5)(p23;q35) resulting in the fusion gene formation. The quantification of NPM-ALK fusion transcripts is a well-established tool for diagnostic purposes and risk stratification during the course of treatment.

**Aims:** Establishment of a multiplex long-range PCR assay identifying patient-specific genomic NPM-ALK fusion sequences for a DNA-based monitoring of minimal residual disease in ALCL patients. Compared to RNA-based methods the quantification of DNA is independent of the gene expression. Additionally, due to the higher stability of DNA, cell-free circulating tumor DNA (cfDNA) should be detectable in the patient’s plasma and may represent a tumor maker for “liquid biopsies” in ALCL patients.

**Methods:** Using a specifically designed multiplex long-range PCR assay, genomic NPM-ALK fusion sequences were identified in 45 ALCL patients. The genomic NPM-ALK breakpoints were analyzed concerning fine structure and breakpoint distribution pattern. Furthermore, the patient-specific genomic NPM-ALK fusion sequences were evaluated for their use as biomarkers in selected cases. For this purpose patient’s blood and plasma samples were quantified using a high sensitive digital droplet PCR assay.

**Results:** In more than 60% of cases the identified breakpoint was localized within repeat regions. The genomic breakpoints within the breakpoint cluster regions of the fusion genes were randomly distributed. Most of the NPM-ALK fusion sequences were characterized by the occurrence of small insertions or deletions indicating the involvement of the non-homologous end-joining (NHEJ) repair system for chromosomal translocation initiation. Using a DNA based quantification assay in a subset of patients, the genomic NPM-ALK fusion gene sequences were detectable in circulating tumor cells in patient’s blood samples as well as in cell-free tumor DNA in plasma samples.

**Summary/Conclusions:** The established multiplex long-range PCR assay is a useful diagnostic tool for the identification of genomic NPM-ALK fusion sequences. This individual tumor maker is independent of gene expression and can be used for therapy response monitoring and relapse detection.

**E1392**

**ARSENIC TRIOXIDE TARGETS BCL6 FOR DEGRADATION AND INHIBITS THE PROLIFERATION OF BCL6-DEPENDENT DIFFUSE LARGE B-CELL LYMPHOMA**

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**Background:** B-cell lymphoma 6 (BCL6) is a transcription repressor and is frequently over-expressed in diffuse large B-cell lymphoma (DLBCL). It suppresses the expression of its target genes ATRX, TP53 and CDKN1A, leading to dysregulation of DNA repair and cell proliferation. It has been shown that BCL6 is an oncoprotein involved in the pathogenesis of DLBCL and represents a potential therapeutic target. Arsenic trioxide (ATO) targets various oncogenic proteins, including PML-RARA in acute promyelocytic leukemia (APL), Tax in adult T-cell leukemia/lymphoma (ATL), cyclin D1 in mantle cell lymphoma (MCL), and NPM-ALK in anaplastic large cell lymphoma (ALCL), for degradation through the ubiquitin-proteasome pathway. ATO is now used for the management of APL, ATL and MCL with proven clinical benefit.

**Aims:** To investigate if ATO targets BCL6 and inhibits the proliferation and growth of BCL6-dependent DLBCL.

**Methods:** BCL6-dependency of a panel of DLBCL cell lines (i.e. OCI-Ly1, OCI-Ly7, SU-DHL-6, OCI-Ly18 and Pfeiffer) was determined based on their sensitivity to proliferation inhibitory activity of the BCL6 inhibitor 79-6 (Calbiochem). The effects of ATO and cisplatin as single agent or in combination on cell viability and apoptosis of DLBCL cells were determined using 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 inhibitor MG132, suggesting that ATO targets 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 NIPA SERINE RESIDUES FOR NPM-ALK-MEDIATED TRANSFORMATION**

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**Background:** Anaplastic large-cell lymphoma(ALCL) is an aggressive non-Hodgkin lymphoma that occurs mainly in children and younger adults. Patients typically show an advanced stage disease as well as an aggressive disease pattern with extraordinary 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 (Illet et al., 2012a). Until now, the specific signal transduction pathway, the crucial phosphorylation sites in NIPA, and the biological role of the phosphosites are still unclear. Molecular insights into the activated pathways of the kinases NPM-ALK may help to identify new druggable targets for therapeutic implications.

**Aims:** In the present study, we investigated the molecular mechanisms as well as the functional impact of the NPM-ALK-induced NIPA phosphorylation.

**Methods:** For this purpose, biochemical methods with ALCL cells were used to examine functional effects of constitutive 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 cell infection of Ba/F3 and primary NIPA-deficient MEL 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 independent NIPA phosphorylation at critical serine residues 354, 359 and 359 leads to dissociation of the inhibitory SCF-NIPA-complex formation. Proteomic-Phosphosite-analyses identified 10 significant upregulated (ratio >2; Log2Fold Change) NIPA phosphorylations upon NPM-ALK expression. Interestingly, 80% of the identified Serine phospho-sites lie within the NPM-ALK binding region of NIPA. This result was further substantiated by generation of a AAΔ310-402 mutant, where NIPA phosphorylation by NPM-ALK was totally abolished. To further prove 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/kit receptor complex formation 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 ALCI.

E1394

APPLICATION OF CELL-OF-ORIGIN SUBTYPES DETERMINED BY DIGITAL GENE EXPRESSION IN HIV-RELATED DIFFUSE LARGE B CELL LYMPHOMAS

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A series of 55 patients with the diagnosis of HIV-related DLBCL infected patients has been scarcely studied.

Methods:

Aims:

In HIV-related lymphomas, COO subtypes were discordantly assigned with Hans and Lymph2Cx assay and COO subtypes showed no impact on outcomes, independently of the method applied.

Summary/Conclusions:

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E1395

CXCR4 AND CXCL12 ARE IMPLICATED IN BONE MARROW INFILTRATION PROCESS OF AGGRESSIVE B CELL LYMPHOMAS AND THEIR INHIBITION SUPPRESSES LYMPHOMA CELL GROWTH IN VITRO

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Background: The chemokine receptor CXCR4 together with its ligand CXCL12 plays a pivotal role in tumorigenesis of solid and haematological neoplasms. Our comprehensive study on the CXCR4 expression in aggressive lymphoma demonstrated that high CXCR4 expression was associated with poor clinical course of aggressive lymphoma patients.

Aims: Therefore, we aimed to comprehensively study the implication of the CXCR4 - CXCL12 axis in bone marrow infiltration process of aggressive lymphoma and to analyse the effects of CXCR4 antagonists on cell growth and migration of aggressive lymphoma cells in vitro.

Methods: To determine whether CXCR4 and CXCL12 expression have any effects on the bone marrow infiltration process of aggressive lymphomas, we performed gene expression analysis on bone marrow biopsies of our diffuse large B-cell lymphoma patient cohort. Therefore, we used 63 bone marrow specimens, whereby 52 bone marrow biopsies were taken at time of diagnosis. Additionally, we generated a novel CXCR4 antagonist -named WK1- by modification of the side chain of AMD070 - a commercially available CXCR4 antagonist. We treated various aggressive lymphoma cell lines (U2932 and RI-1 as ABC-DLBCL and BL2 and Raji as Burkitt lymphoma lines) with CXCR4 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. 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. 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. 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. 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. 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.

Results: The median follow-up of living patients was 8.5 years. IHC studies showed that 48.8% of the cases expressed CD10, 61.5% expressed BCL6, 55.8% expressed MUM1, and according to Hans algorithm 56.6% had a non-GC phenotype. CD30 was expressed in 15.4% of the cases and EBER was found in 21.2%. The expression of MYC was detected in 32.7% of the cases and BCL2 in 44%, and 18% were dual expressers. Rearrangements involving MYC, BCL2 and BCL6 were detected in 26%, 8% and 28%, respectively.

Features associated with shorter OS and PFS were history of AIDS-defining illnesses, HCV-infection and dual MYC and BCL2 expression. For CXCR4 and CXCL12 expression assays were also good prognostic factors for PFS.

Summary/Conclusions:

These data strongly suggest that CXCR4 and its ligand CXCL12 is implicated in the bone marrow infiltration process of diffuse large B-cell lymphomas. Additionally, our in vitro results indicate that treatment of lymphoma cells with CXCR4 antagonists might be a promising new therapeutic intervention to eliminate lymphoma cells.
CLONOTYPE AND MUTATIONAL PATTERN IN TCRγδ LARGE GRANULAR LYMPHOCYTE LEUKAEMIA

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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare heterogeneous T-cell neoplasia whose leukemic cells usually express the αβ T-cell receptor (TCR); only a small subset of cases expresses the γδ TCR denoting the TCРγδ LGLL. Currently, among the different LGL diseases, TCРγδ LGLL remains less studied and several clinical and laboratory data already described in TCRαβ-LGLL have not yet been explored in TCРγδ-LGLL.

Aims: The aims of this work were 1) to characterize TCРγδ-LGLL defining STAT mutational pattern and CDR3 repertoire diversity/clonal composition (clonotype) and 2) to evaluate correlations among LGL phenotype, mutations, TCR rearrangement and clinical presentations.

Methods: In this work 11 patients affected by TCРγδ-LGLL were included. Sanger sequencing was used for mutational analysis on hot-spot regions in the two genes more frequently mutated in LGL disorders, STAT3 and STAT5b. Immunophenotype of LGL clone was defined by flow cytometry analysis. CDR3 repertoire and frequency distribution of TCR gamma gene rearrangements (clonotype) and kind of mutation observed in TCRαβ-LGLL: γδLGL patients with CD16+ TCR αβ LGL and in a group of HIV-infected patients also at one year before diagnosis (N=11) and at complete response (CR) (N=34). EBV expression was studied by in situ hybridization in tumor biopsies. The following clinical and biological parameters were collected from records: age, gender, date of lymphoma diagnosis, ECOG score, extranodal and bulky disease, B symptoms, Ann Arbor stage, serum lactate dehydrogenase and beta2-microglobulin, International Prognostic Index (IPI), HCV and HBV serology, history of opportunistic infection and of AIDS-defining illness, onset of combination antiretroviral therapy, CD4 counts, HIV loads, type and date of response, relapse date, last follow up or death date. McNemar’s test and Wilcoxon test were used to compare quantitative and qualitative variables, respectively. Survival analyses were performed using the Kaplan-Meier method. P-values of less than 0.05 were considered statistically significant.

Results: Our results showed that TCРγδ LGLL had a high incidence of STAT mutations, 9 out of 11 patients carrying STAT3 or STAT5b mutations in a mutually exclusive pattern. At variance from CD8+ TCR αβ LGLL and CD4+ TCR αβ LGLL, TCРγδ LGLL first being more characterized by STAT3 mutations was the latter by STAT5b. TCРγδ LGLL patients were characterized by both the mutations. Thus, TCРγδ LGLL showed features shared by CD8 and CD4 TCRαβ-LGLL. Consistently, TCРγδ LGLL showed the same correlation between immunophenotype and kind of mutation observed in TCRαβ-LGLL: γδLGL patients with CD16+CD56- LGL immunophenotype were characterized by STAT3 mutations (as in CD8+ T-LGLL), while γδLGL patients with CD56+ LGL immunophenotype by STAT5b mutations (as in CD4+ T-LGLL). Moreover, we observed that patients with γδLGLs positive for Vδ2 showed usually indolent course, while Vδ1 was linked to a more symptomatic disease (4 out of 5 symptomatic patients were Vδ1+), whereas no correlation was found between mutational pattern and clinical course. By NGS of TCR gamma gene, we observed that all patients were clonal but two, showing a polyclonal pattern borderline with clonality percentage defined by sequencing kit criteria. Interestingly, these two last patients were the only two patients without STAT mutations. As far as the remaining cases are concerned, among STAT3 mutated patients (n=4), 3 were polyclonal and one biclonal, while STAT5b mutated patients (n=5) were more frequently monoclonal (4/5 monoclonal and 1/5 biclonal). In terms of clonal rearrangements, Vδ3-Jγ1/2, Vδ9-JpD and Vδ8j1/2 were the combination usages most frequently detected. Concerning the clonotype repertoire, CDR3 sequences of the most prominent clonotype present with low frequency in almost all the other γδ patients and two different CDR3 sequences were found shared, each one in two different patients at frequency >10% of the total rearrangements.

Summary/Conclusions: Our data indicate that TCРγδ LGLL can be considered at the intersection of the two types of TCRαβ-LGLL, share with CD4+ T-LGLL mutational features. As already described in TCRαβ-LGLL, also in γδ disease a decreased diversity of TCR repertoire was demonstrated. However, in these γδLGL patients STAT mutations do not correlate with a symptomatic clinical behavior while STAT5b mutations seem to be more frequently linked to monoclonal nature of the LGL lymphoproliferation. Rather, the marker Vδ1 appears to be correlated to symptomatic disease.
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 (ROTY) in the effect of IRF8 on Th17 cell generation. The survival of 67 DLBCL patients diagnosed at the Kaplan–Meier et al. (2012) analysis of the same patient cohort was used to determine if IRF8 correlated with clinical parameters. **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 ROTY 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 ROTY in the DLBCL tumor microenvironment, suggesting that it could be a prognostic factor for DLBCL.

E1399

**GENOMIC PROFILING OF BCL2 AND MYC DOUBLE EXPRESSOR DIFFUSE LARGE B CELL LYMPHOMA**

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**Background:** Diffuse large B cell lymphoma (DLBCL) is an aggressive disease featuring heterogeneous genetic, phenotypic and clinical characteristics. Recently, a negative prognostic impact of double expression of BCL2 and MYC (double expressor (DE) lymphoma) has been identified in several studies. SNP-array (SNP-A) studies have already led to the identification of novel genomic aberrations in ABC and GCB subtypes of DLBCL whereas similar analysis has not been done in DE and non-DE DLBCL.

**Aims:** To characterize the landscape of genomic aberrations in DE and non-DE DLBCL groups using SNP-A and interphase fluorescence in situ hybridization (FISH).

**Methods:** Immunohistochemical and FISH analysis was performed on tissue microarray of formalin fixed paraffin embedded (FFPE) tumor tissue samples using Bcl2 (124, DakoCytomation) and MYC (Y69, Epitomics) antibodies and FISH MYC (Zytovision), Bcl2 (Abbott/Vysis), Bcl6 (Abbott/Vysis) break-apart probes and MYC FISH (Zytovision) double fusion probe. Infinium HD whole-genome genotyping assay with the HumanCytoSNP SNP-FPPE-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 Bcl2 in 90% (56 cases) and MYC in 91% of cases (56 cases) were informative for MYC, 56 cases for Bcl2, and 65 cases for Bcl6. 7 cases (11.4%) were positive for MYC translocation, 14 (25%) for Bcl6, and only 3 (4.6%) were positive for Bcl2. No cases of FISH MYC and bcl2 double positive DLBCL were identified. Genomic DNA from FFPE tumor tissue for SNP-A was available in 66 cases (56%). SNP-A results were informative in total 329 genetic aberrations in about 60% of patients (pts) with diffuse large B-cell lymphoma (DLBCL). Pts available in 66 DLBCL cases. SNP-A analysis detected in total 329 genetic aberrations per case (~5 aberr./case) and shared the most common aberrations in 66 cases, respectively. Cases with MYC positive

**Summary/Conclusions:** Our findings suggest that IRF8 expression in the tumor microenvironment inhibited the generation of Th17 cells through its antagonistic effect on ROTY in the DLBCL tumor microenvironment, suggesting that it could be a prognostic factor for DLBCL.

E1400

**ARQ 531, A REVERSIBLE BTK INHIBITOR, DEMONSTRATES POTENT ANTI-TUMOR ACTIVITY IN ABC-DLBC AND GCB-DLBC**

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**Background:** B-cell receptor (BCR) signaling has emerged as a critical pathway for B-cell lymphoma development. BTK, a key mediator of BCR signaling, is a major target for ibrutinib. Ibrutinib has demonstrated efficacy in chronic lymphocytic leukemia (CLL), mantle cell lymphoma and Waldenström macroglobulinemia. However, as anticipated by preclinical models, clinical objective response rates of only 37% in ABC and 5% in GCB diffuse large B cell lymphoma (DLBCL) were reported. ARQ 531 is a potent reversible inhibitor of BTK, highly effective in targeting BCR signaling. Kinase profiling indicated that ARQ 531 is differentially selective in DLBCL vs non-DLBCL regimens with inhibition of HCK and BLK kinases. ARQ 531 caused significant growth inhibition (GI50=1 µM) of hematological malignant cell lines and showed greater efficacy than ibrutinib in a CLL mouse model.

**Aims:** We aim to assess biological and anti-tumor effects of ARQ 531 in in vitro and in vivo models of B-cell lymphoma.

**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 tumor 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 residence time (65 min). Inhibitor exhibited strong anti-proliferative activity in TMD8 (GI50=0.13 µM) and SUDHL-4 (GI50=0.02 µM) cell lines. Ibrutinib, 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 93% after 14 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 group.

**Summary/Conclusions:** ARQ 531 is a potent reversible inhibitor of BTK. Its disease 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 models 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 is not largely unique. Our findings are consistent with this view.

**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 pts resistant to R-CHOP. We are reporting update data of an interim analysis on the first 80 enrolled pts.

**Methods:** 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 a 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 sur-
vival (OS). The efficacy of R-CHOP was evaluated according to Cheson criteria by performing standard hematochemical and instrumental (TC and FDG-PET) tests and defining complete remission (CR), partial remission (PR), non response or progressive disease (PD). Genomic DNA was extracted from peripheral blood of 80 pts. SNPs analysis was performed by an Affimmix array. To date, 21 SNPs from 19 candidate genes (ABC21, ABC1, ABC22, ABC02, CYB5A, CYP3A5, FABP5, FGF2, FGFR2A, GSTP1, IGF, MARCKS, IL1H1, NCF4, NOQ1, NOQ2, RAC2, TNF, TOP2A, TP53, TUBB) involved in pharmacokinetics and pharmacodynamics of R-CHOP (www.pharmkg.org) selected and analysed in relation to R-CHOP efficacy. Univariate and multivariate logistic regression analyses were performed to evaluate associations between SNPs and clinical/pathological characteristics or survival parameters (PFS and OS).

Results: Median age was 63 years. There were 37 men and 43 women. 47.5% of pts were in stage I-II, 52.5% of pts in stage III-IV. 27.5% of pts had bulky disease, 43.8% of pts had involvement of extranodal site. 47.5% of pts had pathological LDH value. According to the revised IP, 15% of pts were in the low risk group, 58.7% in the intermediate, and 28.3% in the high risk group. 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 FGR2A rs1801274 as a predictor of PFS (p=0.045). Pts with HR or RR genotypes showed shorter PFS than pts with HR genotype (HR: 2.43, 95% CI: 1.02-5.82). No statistically significant correlation was found between SNPs and OS.

Summary/Conclusions: Our preliminary data obtained in a limited number of pts, show an association between a SNP of the low affinity FGR2A gene involved in the activity of rituximab and PFS. Further insights will derive from the completion of this project accrual to reach the planned number of cases at the end of our study.

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E1402

CDK4/6-INHIBITION BY ABEMACICLIB INDUCES POTENT EARLY G1-ARREST IN MCL CELL LINES AND SHOWS SEQUENCE-SPECIFIC INTERACTIONS WITH CYTARABINE AND IBRUTINIB

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Background: Mantle cell lymphoma (MCL) is characterized by t(11;14) resulting in a constitutive cyclin D1 overexpression. The cyclin D1-CDK4/6 complex inactivates Rb through phosphorylation, leading to G1/S-phase transition. Therefore, inhibition of CDK4/6 is an efficient and rational approach to overcome cell cycle dysregulation in MCL.

Aims: We evaluated the efficiency of the novel CDK4/6 inhibitor abemaciclib in various MCL cell lines and in primary MCL cells in combination with cytarabine (AraC) and ibrutinib.

Methods: MCL cell lines (Granta 519, JeKo-1, Mver-1, Mino) and primary MCL cells were exposed to abemaciclib alone and combined with AraC or ibrutinib. In some experiments, cells were pretreated with abemaciclib and exposed to AraC or ibrutinib with or without consecutive wash-out of the CDK4/6 inhibitor. Proliferation and viability were measured by trypan blue staining and Cell Titer Glo assay. Combination Index (CI) to assess synergy or antagonism was calculated using the Fractional Product method by Webb (1963). Flow cytometry was applied for cell-cycle (PI-staining) and apoptosis analysis (Annexin V PE/7AAD-staining). Protein expression and phosphorylation status of various downstream proteins was analyzed by Western Blot analysis.

Results: Abemaciclib inhibited cell proliferation by induction of early G1-arrest. We observed an almost complete and reversible G1-arrest in all sensitive cell lines by FACScan analysis (JeKo-1: G1-phase +51.7%, S/G2-phase -51.7% at 31.25 nM after 24 h; G1-phase +35.4%, S/G2-phase -38.4% after 72 h), whereas cell viability was not reduced. IC50-values of sensitive cell lines (JeKo-1, Mver-1, Mino) were <30 nM after 72 h. Western Blot analysis revealed reduced phosphorylation of Rb on serine 795 without changes in CDK 4 and cyclin D1 expression. Pretreatment with abemaciclib in a concentration of 31.25 nM after 24 h resulted in synchronized S-phase entry in all sensitive cell lines (e.g. Mino: G1-phase -20.4%; S-phase +30.5%). Accordingly, sequential combination of abemaciclib followed by AraC showed strong synergy in Mino cells (CI=0.22 for 31.25 nM abemaciclib / 3.3 µM AraC). In contrast, simultaneous exposure to both agents had a protective effect against AraC treatment in all sensitive cell lines, due to an ongoing G1-arrest (Mino: CI=0.19 for 31.25 nM abemaciclib / 3.3 µM AraC). Sequential administration of abemaciclib and ibrutinib had synergistic or additive effects in sensitive cell lines (CI: JeKo-1=0.24; Mver-1=0.19; Mino=0.03 for 31.25 nM ibrutinib / 2.5 µM Ibrutinib), whereas the simultaneous administration of both showed additive effects at most (CI: JeKo-1=0.1; Mver-1=0.1; Mino=0.09 for 31.25 nM ibrutinib and 2 µM Ibrutinib). In primary MCL cells abemaciclib had no impact on cell death or sensitization since no cell proliferation was observable and cells where resting in G1-phase.

Figure 1.

Methods: The study included 26 patients (15 males and 11 females; age 36-92, median 67) with the diagnosis of AITL established on the basis of WHO 2008 diagnostic criteria. LN, BM and peripheral blood lymphocytes were tested for T-cell clonality according to BIOMED-2 protocol with subsequent fragments analysis on ABI PRISM 3130 (Applied Biosystems). The material was examined at the diagnosis and at various points of patient’s treatment. In 5 patients selection of CD8+ and CD4+ populations of PB lymphocytes was performed with MidiMACS and MiniMACS Separators using CD4+ and CD8+ MicroBeads (Miltenyi Biotec). In 4 patients, traditional TCR-γ/δ chain gene rearrangements from those detected in the bone marrow (BM), peripheral blood (PB) and other tissues, T-cell clonality testing itself may not distinguish between neoplastic or reactive lymphoproliferation in the BM and PB. Therefore, T-cell clonality of CD4+ and CD8+ populations of peripheral blood lymphocytes in patients with AITL had been tested during the course of disease.

Aims: To determine immunological characteristics of persisting in the PB and BM T-cell clones in AITL patients.

Results: In 21 of 26 patients (80.7%) at least one of the clonal products isolated from the BM and/or PB mismatched the clonal products isolated from the LN. In 12 patients (46%) clonal products found in BM and PB were different from those identified for LN. Thus, at the diagnosis 14 of 20 patients (74%) had PB and BM T-cell clones distinct from LN T-cell clones. 14 of 20 patients T-cell clones of PB and BM were tested repeatedly during the course of disease treatment. In 7 of 14 patients (50%) clonal rearrangements persisted for a long time and do not disappear upon reaching the remission of the disease. The observation period averaged 12 months (1 to 44 months). No correlation of T-cell clones persistence and the activity of EBV infection in the PB was found. Selection of CD4+ and CD8+ T-lymphocyte populations was performed for 4 patients in remission with persistent T-cell clones. In all cases,
Clonal products, which were originally identified in the BM and PB were shown to belong to the CD3+ population of cells (Fig.1). In one case, BM and PB derived CD4+ cells also shared a clonal product with LN cells tested at the diagnosis. In this case, CD4+ population selected from PB cells at the diagnosis carried clonal rearrangements, fully consistent with that of LN.

Summary/Conclusions: One may conclude that CD5+ T-cell clones identified in the BM and PB only match those identified in LN and are all found within patients with AITL (76%). These clones can persist for a long time (the period of observation from 1-40 months), may not disappear in remission and probably have reactive nature. Therefore exclusive T-cell clonality in PB and/or BM should not be treated as minimal disease or relapse in AITL.

E1404
CD5 POSITIVE DIFFUSE LARGE B CELL LYMPHOMA SHOWED FREQUENT MYC EXPRESSION AND AGGRESSIVE CLINICAL BEHAVIOR
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Background: Aberrant expression of CD5 distinguishes a unique immunohistological subtype of diffuse large B cell lymphoma (DLBCL). This CD5+ DLBCLs, either de novo lesions or transformed from preceding low grade lymphomas, may only be identified in LN for the majority of patients with AITL. The incidence of CD5+ DLBCL was variably reported from 5-22% of all DLBCLs in western countries and Japan, however, no exact data available in Koreans.

Aims: This study aimed to investigate clinicopathologic features of CD5+ DLBCL in Korea.

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 BCL2, BCL6, CD5, CD10, CD23, D30, IRF4/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 suspected in 7 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, BCL5, IRF4/MUM1 and Ki-67 (all p<0.05). Double expression of both BCL2 and MYC was found in 9 of 30 cases (30%). Also, CD5+ DLBCL showed more frequent bone marrow involvement, advanced stages and high international prognostic index (all p<0.05). In univariable survival analysis, CD5+ DLBCL revealed significantly shorter progression free survival (median 8.2 months) compared with DLBCL-NOS (median 56.3 months) (p<0.05)

Summary: The present study confirms the aggressive nature of CD5+ DLBCL in Korea. The incidence, clinical presentation, and pathologic features including cell of origin coincide with previous reports from western population or Japanese. However, frequent high expression of MYC without chromosomal structural alteration was a unique finding in our study. Expression of CD5 should be routinely investigated in DLBCL to find this particular aggressive subtype.

E1405
REACTIVE FLORID B-LINEAGE LYMPHOID PROLIFERATIONS IN HIV INFECTION MAY MIMIC LYMPHOMA
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Background: Approximately 7 million people are living with Human Immunodeficiency Virus (HIV) infection in South Africa (SA) (2015), which is associated with an increased risk of lymphoma. Although there is limited local information available, previously published data from the Johannesburg academic complex of hospitals (SA) showed an HIV prevalence of >90% in patients diagnosed with high grade B cell non Hodgkin lymphoma (NHL) who were tested for HIV (n=568), during the period 2007-2009. The diagnosis of lymphoma with comorbid HIV infection is, however, challenging as lymphomas may present with reactive conditions which may mimic lymphoma (such as Tuberculosis (TB)). Within this setting reactive B lineage lymphoid proliferations (RBLP) in the blood and bone marrow may raise a differential diagnosis of lymphoma.

Aims: This study aims to document the clinicopathological features of florid RBLP in the setting of HIV infection in order to provide an approach to differentiating reactive and clonal processes.

Methods: A retrospective database search was performed of the laboratory information system (National Health Laboratory Service) that screened pathologic reports for samples referred to the Departments of Molecular Medicine and Haematology and Anaesthesiology at the Johannesburg Academic Complex during 2007-2011, supplemented with results of immunophenotypic analysis from 2007-2016. Demographic and clinicopathological findings were collected for patients identified with florid RBLP who showed no definite evidence of monoclonality.

Results: During this period, 38 patients were diagnosed with florid RBLP with up to 70–80% of cells in blood or bone marrow comprising reactive B cells (including mature B, plasma blasts and plasma cells). All patients tested were HIV positive, with a median age of 28 years (range 6 months-79 years). There was a bimodal age pattern with a peak in children <1 year of age (34% of patients) and the second peak in the elderly (33%). A majority of these lymphomas is virtually absent in children under a year of age. Common clinical presentations included cytopenias (85%); infection (70%) (commonly Cytomegalovirus (35%), TB (30%) and bacterial septicaemia (22%)); hepatosplenomegaly (42%); and lymphadenopathy (36%). Patients showed increases in serum total protein levels (reflecting hypergammaglobulinaemia), with increased inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) and evidence of increased cell turnover (high uric acid, B2 microglobulin and lactate dehydrogenase levels). Extremely high HIV viral loads (VL) were documented (median 1 612 003 copies/mL, range 12 000 - 10 000 000). In one patient VL was virologically suppressed. This significantly different from lymphoma patients where median VL ranged from 16 000-97 000 dependent on subtype. Median CD4 counts were also higher in this subgroup of patients when compared to patients with lymphoma (see table 1).

Summary/Conclusions: In the setting of HIV, reactive conditions may mimic lymphoma and vigilance is needed in the confirmation of monoclonality. Patients with RBLP presented at a younger age when compared to their counterparts with lymphoma. They had extremely high VL with higher CD4 counts, suggesting this may be a feature of early HIV disease and the possibility of a seroconversion type illness should be considered.

E1406
MICROVESSEL DENSITY IN CD30 POSITIVE DIFFUSE LARGE B-CELL LYMPHOMAS
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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common and one of the most homogeneous lymphomas. Therefore, it is critical to further stratify cases of DLBCL into biologically similar and clinically meaningful subgroups, which will not only guide prognostic assessment and facilitate therapeutic decisions but also stimulate further research to understand the pathobiology and develop novel therapies to the lymphoma. The current reporting of a number of different kinds of tumors have indicated that microvesSEL quantification may be useful in predicting disease outcome.

Aims: The aim of this study was to examine the relationship between microvesSEL density (MVD) as a parameter of tumor angiogenesis, and the immunohistochemical subtypes with lymphoma and unclassifiable lymphomas with intermediate features CL among Koreans.

Methods: We retrospectively identified cases of DLBCL diagnosed between January 2010 and January 2016 at our Institution. The following large B-cell lymphoma subtypes were excluded from this analysis: post-transplant lympho-proliferative disorders with a DLBCL morphology, Primary Mediastinal large B-cell lymphoma and Burkitt’s lymphoma. A total of 30 cases of CD5+ DLBCL were retrieved among 100 cases of DLBCL.

Results: Limited follow-up data was available, with only 8 patients documented to be limited follow-up data was available, with only 8 patients documented to be 22 nd Congress of the European Hematology Association
Microvascular 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).

In patients with CD30− DLBCL treated with R-CHOP were found a smaller number of vessels compared with patients CD30 positive (fig.1, p < 0.05).

**Figure 1.**

**Summary/Conclusions:** CD30 is expressed in approximately 29% of all DLBCL and defines a novel subgroup of diffuse large B-cell lymphoma with a more favorable prognosis. Microvascular density expression is lower in CD30 positive DLBCL. The advent of brentuximab vedotin and its well-established effectiveness in other types of relapsed lymphomas has introduced the possibility of its application in this subset of patients.

E1407

**ANTIGEN SELECTION PROMOTES CLONAL CYTOTOXIC T-CELL RESPONSES: HIGH-THROUGHPUT IMMUNOGENETIC EVIDENCE**

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**Background:** Background of T-LGL 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 chimerism and tested negative for Bcr-Abl transcripts. TRBV-TRBD rearrangements were amplified on gDNA and subjected to paired end NGS, covering the CDR3 twice/sequence.

To increase the consistency of results, raw NGS reads were analyzed by a purpose-built bioinformatics algorithm, performing: (i) quality filtering, (ii) merging of filtered in paired reads and (iii) quality filter of stitched sequences. Filtered-in sequences were submitted to IMGT/HighV-QUEST, and metadata was processed by an in-house dedicated bioinformatics pipeline.

**Results:** Only productive TRBV-TRBD 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 investigated include: (i) pronounced skewing of the TRBV repertoire, representing the presence of more than one immunodominant clonotype; (ii) in the analysis of longitudinal samples from the son, persisting clonotypes albeit with fluctuating frequencies (clonal drift); and, (iii) shared (‘public’) clonotypes between father and son. In the T-LGL leukemia of donor origin, the immunodominant clonotype was detected amongst the polyclonal donor repertoire and subsequently expanded in the recipient, persisting over time and accompanied by a few other considerably expanded, albeit smaller, clonotypes.

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 167 BM samples: 167 were analyzed by both nested polymerase chain reaction (PCR) and real-time quantitative PCR (RQ-PCR). The sensitivity of each molecular analysis was tested 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.

Results: The sensitivity and the accuracy of each molecular analysis was tested, reaching a uniform sensitivity of 10-5 and a quantitative range for RQ-PCR of at least 10-3. Ninety-three/114 FL BM samples were submitted to sequencing (75/93) being PCR+/RQ-PCR+ or PCR-/RQ-PCR- in all the 4 labs. The remaining 18/93 (19%) were alternatively positive and negative in the inter-lab evaluations, representing samples with very low MRD levels, thus defined as “borderline” (brd): 6/18 were PCR-brd/RQ-brd; 6/18 were PCR-brd/RQ-positve not detectable by RNQ and 6/18 were PCR-RQ-brd; 6/18 were PCR-RQ-PNQ; and 6/18 were PCR-RQ-RQ-negative. Overall, considering the 167
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, hyperglobulinemia, and autoimmune manifestations. Interpretation of histological and immunohistochemical data can be difficult due to the small number of tumor cells in biopsies, surrounded by abundant polymorphonuclear 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, blood, bone marrow and skin of AITL patients with corresponding T cell clonality results.

Methods: Lymph nodes (LN), skin biopsies, blood and bone marrow (BM) samples were studied for 40 patients with AITL. The male/female ratio was 25/15, median age was 65 years (36-92). To evaluate T-cell clonality rearranged TCRγ and TCRβ gene rearrangements were PCR-amplified according to BIO-MED-2 standardized protocol and analyzed by capillary electrophoresis on ABI PRISM 3130 (Applied Biosystems). Sensitivity of T-cell clonality assay was limited to 10% of clonal T-cells of the total T-lymphocytes in the sample. Gly17Val mutation was analyzed by quantitative allele-specific (qAS) TaqMan Real-Time PCR assay. The detection level of this method was 1% of mutated cells in the total cell population.

Results: The clonal TCR gene rearrangements in LN were found in 37 of 40 patients (92%). RHOA (Gly17Val) mutation in LN was revealed in 60% (24 of 40) patients. T-cell clonality was detected in 26 of 28 primary samples of BM, but in 12 of 26 patients (46%) clonal TCR rearrangements were not matched in length with rearrangements detectable in LN. Number of cells with RHOA mutation was highest in the LN (in average 26.7% of the total cells), while in the bone marrow RHOA mutation was undetectable (in 7 patients), or detected in 10 patients in a small amount (in average 2% of the total cells). Combined historical investigation, T-cell clonality and RHOA (Gly17Val) testing showed BM lesion in 76% of patients (13 of 17) with at least one of the methods. Blood and bone marrow samples examined simultaneously showed slightly higher numbers of RHOA positive cells in the blood than in the BM in 5 of the 7 RHOA positive patients. Significant percentage of cells with a RHOA mutation (in average 25% of the total cells) was revealed in 5 of 6 skin samples from RHOA positive patients. We have found good correlation (Spearman’s Rho=0,8198, p-level <0,00001) between T-cell clonality (matching with LN clonal peaks) and the number of RHOA positive cells in the AITL tissues (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 CHITOSIDROSIDE ACTIVITY, CCL18/PARC, 7-KETO-CHOLESTEROL AND GLUCOSYLPHOSPHINGOSINE CONCENTRATIONS FOR SCREENING OF LYSOSOMAL STORAGE DISORDERS

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Background: Gaucher (GD), Niemann-Pick Type A/B (NPA/B), Niemann-Pick Type C (NP-C) and Gaucher-like 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 Chitosidroside activity (ChT), CCL18/PARC, 7-ketocholesterol (7KC) and glucosylphosphingosine (Lyso-Gb1) concentrations in previously mentioned LSDs.

Methods: ChT activity, CCL18/PARC and 7KC concentrations were measured in 146 plasma samples from subjects with suspected LSD (32 GD, 7 NPA/B, 90 NP-C and 17 LALD) received in our laboratory. In addition, a new biomarker, the Lyso-Gb1 concentration, was evaluated in 83/146 of previous mentioned subjects, 19 of them with confirmed LSD diagnosis. ChT was evaluated using a fluorogenic substrate, CCL18/PARC concentration by ELISA and 7KC and Lyso-Gb1 by liquid chromatography followed by tandem mass spectrometry.

Results: A total of 9/32 (28%) samples with suspected GD showed high ChT activity, 32/90 (35.5%) with NPA/B, 3/17 (17.6%) with LALD and in the rest the ChT activity was normal. Among the 19/90 (21%) with suspected NP-C and 2 carriers of NP-C only 3/43 (7%) with suspected NPA/B and altered biomarkers were confirmed. Among the 23/90 (26%) with suspected NP-C and some elevated biomarker four were diagnosed of NP-C, and two carriers showed some biomarker higher than cutoff. Of the 8/17 (47%) referred to LALD suspicion with some elevated biomarker six were affected. All GD confirmed patients show high levels of Lyso-Gb1 whereas none of the other cases showed elevation for mentioned biomarker.

Summary/Conclusions: The screening of three biomarkers: ChT activity, CCL18/PARC and 7-ketocholesterol (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 divided into a primary, genetic form and a secondary, acquired form. It is 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 soluble IL-2 receptor alpha subunit (sIL-2Rα) 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%) with “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 suspected 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 papillodema (discovered during workup for suspected autoimmune disorder). The second patient, an 11 months old “CNS-HLH positive” patient (with history of a year of “CNS-HLH” 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 high 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 high risk genetic forms like Griscelli syndrome, that sIL2Rα is locally produced in the CSF secondary to cellular infiltration of CNS-HLH, that sIL2Rα can allow the unambiguous identification of all the GD patients but is not useful for the other LSDs.
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 normalisation, partial remission (PR) as platelet count doubling and ≥30% increase, 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 (18-66) 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 related to malignancy. Proportionate to incidence of secondary TTP, 85% required immunosuppressive therapy and rituximab. Although the final remission rates and range were: Hb 10.5±3.38 g/dl (4–14.5 g/dl), WBC 6,233±3,593/mm3 (2,800–62,000/mm3), LDH 372±302 U/L (226±1,162 U/L), bilirubin 1.8±1.78 mg/dl (0.3-8.8 mg/dl), DAT was positive for IgG - IgG/C3d in 13 patients (92.9%) and for IgG/IgA in one patient (7.1%). As to immunology, CD3+CD4+/CD8+ ratio and/or anti-TG in 3 (21.4%) patients. In 10 (71.4%) cases simultaneous, whereas in 4 cases (28.6%) sequential cytopenia presence were reported. All patients presented with thrombocytopenia, all with positive DAT - but only 8 (57.1%) with Hb<11g/dl-, 7 (50%) with leucopenia and 6 (42.8%) with neutropenia. Severe presentation was reported at disease onset in 10 (71.4%) patients. All patients received IVIG (1-6 g/kg) and high dose methyl-prednisolone (30mg/kg/h) – one or more times depending on clinical course. Prednisolone was administered in 10 (71.4%), cyclosporine in 8 (57.1%) and vincristine in 1 patient. Mean follow-up was 5.4 years (18 months - 13 years), during which 5 (35.7%) patients presented with one or more complications related to treatment: Cushing syndrome, osteoporosis, hypothyroidism, renal dysfunction and/or peripheral neuropathy. No severe infection or death was reported during the 15 year period. Disease relapses (1-3) were reported in 8/14 (57.1%) patients. With regards to outcome, 8/14 (57.1%) remained in LCR, 1 (7%) 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 cytopenias 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 IVIG (1-6g/kg) and PO PO 1mg/kg once daily. 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 ≥10 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. Mean age was 32 years (range 18-42), 6 were female. Median Hb at diagnosis was 5.8 g/dL (range 4.8–6.2 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 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 8-40). One patient remained steroid-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 2016. These results may be comparable to standard doses of rituximab, with a lower cost, and deserves further inquiry. The emergence of additional autoimmune phenomena (SLE, Evan’s syndrome) is unpredictable and can be an obstacle for appropriate data analysis in prospective AIHA studies.

E1417

NEW EPO-RECEPTOR MUTATION IN A 17 YEAR OLD WOMAN WITH ERYTHROCYTOSIS
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Background: Erythrocytosis is defined when red cell, hematocrit (Hct) and hemoglobin (Hb), are elevated above normal limits. Causes of erythrocytosis can be primary and secondary. Secondary causes are divided into congenital and acquired. There is a group of patients with idiopathic erythrocytosis. Aim: 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%. There was a history of consanguineous marriage in 26 of the families (74%). 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 hypertiglyceridemia were present in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (85.7%). Hypofibrinogenemia was detected in all patients. All patients had neutropenia and thrombocytopenia. Hypoperfettinemia 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 (%25.7). The overall mortality rate was 57% (20 cases) in our series. Twenty children died opportunist infection (n=10) or of disease progression (n=10).

Results: The test revealed a new EPO-receptor mutation (c.1275_1290dup), which had never been described before.

E1418

FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHILDREN
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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder characterized with uncontrolled activation of T-helper lymphocytes and macrophages and over-release of inflammatory cytokines. The only curative treatment is hematopoetic stem cell transplantation (HSCT). Aim: 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 hypertriglyceridemia were present in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (85.7%). Hypofibrinogenemia was detected in all patients. All patients had neutropenia and thrombocytopenia. Hypoperfettinemia 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 (%25.7). The overall mortality rate was 57% (20 cases) in our series. Twenty children died opportunist infection (n=10) or of disease progression (n=10).

Figure 1.
ABNORMAL MONOCYTE POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

Methods:

Aims: Monocyte subpopulations display a prominent role in innate immunity but also mediate pro-inflammatory responses and T-cell activation. The monocyte subsets in CIN patients were studied in CIN. 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 monocytes CD14+/CD15/DR54/lowCD33/+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±549/µl and 142±130/µl, respectively (range 200-1800/µl and 700-200/µl, respectively). The proportion of classical CD14++/CD16- cells was significantly decreased in CIN patients (79.60±7.60%) compared to the healthy individuals (87.90%±3.70%) (P<0.0009). In contrast, a significant increase was observed in the proportion of CD16 positive cells in CIN patients (16.81%±6.75%) compared to 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/DR54/lowCD33/+CD11b+ MDSCs was significantly increased in the patients (6.18%±3.92%) compared to the healthy controls (0.34%±0.52%) (P<0.0412).

Summary/Conclusions: CIN patients displayed 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/DR54/lowCD33/+CD11b+ MDSC in CIN may simply reflect a compensatory reaction aiming to suppress the T-cell activation. Isolation of the above cell populations and transcriptome studies are currently in progress in our laboratory.

References

Background: Autoimmune hemolytic anemia (AIHA) is not commonly seen in childhood, and is extremely rare in infancy. Absence of guidelines renders management of the disease difficult in children – and even more so in infants.

Aims: Aim of the report is to present a number of cases of infantile AIHA, refractory to conventional treatments, demonstrating response in administration of rituximab.

Methods: The report concerns four infants (3 baby girls and one baby boy) who presented with AIHA. Data regarding demographics, personal and family medical history, immunologic assessments, previous treatments and response to rituximab were studied.

Results: Age at diagnosis of AIHA was 4-6 months. In 3 cases (cases number 1, 2 and 3) personal and family history, as well as laboratory screening at diagnosis, did not reveal presence of any other hematologic, autoimmune or immunologic condition. In case number 4 AIHA followed the diagnosis of giant cell hepatitis. Hospitalization before rituximab administration ranged between 1 and 3 months and multiple transfusions, administrations of intravenous immunoglobulin (maximum dose 6g/kg), repeated doses of intravenous methyl-prednisolone (30mg/kg) followed by oral prednizolone (max 5mg/kg), all failing to achieve sustained response. Rituximab was administered at 370mg/m² in 4 weekly infusions. In 3 infants 5 monthly infusions followed. Stabilization of hemoglobin and improvement of hemolysis parameters were observed after the 3rd-4th weekly infusion in all infants. In 3 patients (no 1,2,3) CD19+ and CD20+ B cell assessment before and after rituximab administration was performed. Complete elimination (<1%) was observed in all patients after the 1st-2nd infusion. Despite B cells returning to normal 11 months after treatment, infant no 1 remained in clinical remission during follow-up (22 months post treatment). Infant no 2 remained in clinical remission for the 16 month post treatment follow-up, despite B cell normalization. Infant no 3 relapsed following B cell normalization, 11 months after rituximab administration. Infant no 4 did not undergo B cell measurements and relapsed one year after completing rituximab therapy. The 2 patients that relapsed were re-treated with 4 rituximab infusions: patient no 3 remained well for the 18 month follow-up, whereas patient no 4 remained well for 10 years – again relapsing and receiving her 3rd rituximab treatment with good response for the remaining 7 month follow-up. None of the patients presented with adverse reactions during the infusions or with relevant infections as 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.

E1424

EARLY LESSONS FROM WHOLE-EXOME SEQUENCING IN THE CLINICAL DIAGNOSIS AND MANAGEMENT OF RARE INHERITED ANEMIAS


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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 with high 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. Furthermore, WGS is likely to lead to the identification of novel genes involved in pathogenic and normal erythropoiesis.

Aims: In the last 12 years we have undertaken 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 analyses 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 the parents have a rare anaemia 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 Stumpy for read alignment, Platybus for variant calling and Ingenuity Variant Analysis (Qiagen) for variant annotation and filtering, followed by manual verification and classification.

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 erythropoietic 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 level, and positive C. 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 a low platelet count which was associated with bleeding. They also required a higher number of plasma exchanges to recover. Five patients relapsed, 4 with low ADAMTS13 level. 4 patients were splenectomized and received immunomodulators. One patient received only plasma exchanges when relapsed. One patient died immediately after diagnosis before receiving plasma exchange. HUS group patients had higher creatinine level which was associated with oliguria and dialysis requirement. Neurological symptoms were more frequent as well. Two patients progressed to renal failure and one was transplanted. Two other patients received eculizumab and 1 relapsed when treatment was interrupted during pregnancy. sTMA patients showed more cardiac events and fever. Main triggering causes were: 6 malignant hypertension, 5 systemic lupus erythematosus, 4 neoplasia, 3 pancreatitis, 2 pregnancy, 1 tuberculosis, 1 glomerulonephritis, 1 dermatomyositis. Six patients died (4 cancer related). In the multivariate analysis, high LDH level was significantly associated with relapse (p=0.012) while the number of schistocytes showed a trend to statistical significance (p=0.063).

Summary/Conclusions: ADAMTS13 determination is a useful tool in TMA differential diagnosis. A high LDH level, and also probably the number of schistocytes, could be valuable to predict relapse in TMA patients.

E1427

CHILDREN WITH CHRONIC–REFRACTORY AUTOIMMUNE CYTOPENIAS: A SINGLE CENTER EXPERIENCE

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Background: Autoimmune cytopenias are a group of heterogeneous disorders characterized by immune-mediated destruction of one or more hematopoetic lineage cells. They can be idiopathic or occur as a manifestation of other underlying disorders, such as autoimmune diseases, immunodeficiency, autoimmune lymphoproliferative syndrome, tumors, medications or infections.

Aims: The aim of this study was to evaluate the clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children followed up at our hospital.

Methods: A total of 337 files of information belong to patients with chronic or refractory autoimmunity 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–three of the patients with chronic autoimmune cytopenias (6.8%) had an immune deficiency or an autoimmune disease. The median age of diagnosis was 3.1 years (between 6 months–16 years) and the ratio of male/female was 1.3. The median duration of following was 2.6 years (between 4 months and 18.5 years). A total of 13 patients (56.5%) had single- lineage cytopenias and 10 (46.5%) had multi-lineage cytopenias. Shows last diagnosis of the patients. In 10 of the patients, first cytopenias were more 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, azathioprine, cyclosporine A, aza thioprine, and danazol. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primer disease. That diseases were diagnosed as systemic lupus erythematosus in 4 patients, hypogammaglobulinemias in 3 patients, and celiac disease in 1 patient. Cytopenias have been in 14 of the patients. One patient had been diagnosed as CVID died.

Summary/Conclusions: Cytopenia may be the first finding of an immunodeficiency or autoimmune disease and primary disease may be diagnosed in the clinical course. Early diagnosis is important because of beginning to the early treatment of underlying disease.
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 Cytology, Karolinska University Hospital. The patients were diagnosed and treated for HM-HLH at the Hematology Center of the same hospital, between 2009 and 2016. Tissue samples (bone marrow, peripheral blood, lymph nodes) were analyzed according to standard procedures, using monoclonal antibodies (BD, DAKO, Beckman Coulter, BioLegend). Cells were acquired using 4-color Canto A or 8-color Canto II cytometers (BD), and analyzed with BD FACSDIVA software. Neoplastic clones of myeloid or lymphoid character were excluded from reanalysis for the purpose of this study. Bone marrow samples were obtained from 31 patients shortly before and from 24 patients following HLH-diagnosis; in 13 patients paired BM samples were available.

Results: Patient characteristics are presented in table 1. Bone marrow B-cell lymphopoenia 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 below 200 cell/mm³ 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 lymphoid tumors before HLH diagnosis 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 at HLH onset (40% vs 31%), although it was of non-neoplastic 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 may reflect disease-specific impact on BM microenvironment. Further study will be carried out to confirm findings in a large, possibly prospectively collected patient group. Control group of patients with respective malignancies but without HLH will be included.

Platelets disorders

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. The purpose of this study was to 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 30x10⁹/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 IVg (43%) were the most used drugs followed by rituximab (26%) among those who were treated. Romiplostim (15%) and Eflotrombopag (9%) are used too but not anymore than mycophenolate (18%) and azathioprine (22%). Seventeen 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. Patients aged more than 60 years old were less likely to experience a bleed than older adults (>70 years), who were most at risk. Platelet counts, expectedly, was associated with bleeding with those presenting with a platelet of <30x10⁹/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.

A multicentre, single arm open label study evaluating the efficacy and safety of eltrombopag in patients with severe persistent immune thrombocytopenic purpuria (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 (IVg) are often committed to splenectomy or prolonged immunosuppression. Splenectomy is potentially curative 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 ITP with platelet counts of <30x10⁹/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 IVg to maintain a platelet of ≥30x10⁹/L within 6 months of diagnosis. Prior splenectomy was not a requisite.
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 reduced 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 steroid 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 ≥50% 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 54 (40, 64) years, and median (Q1, Q3) platelet count was 113 (55, 293)x10^9/L. Of the 29 patients with ITP diagnosed ≥6 months, 18 (62%) had a history of antiplatelet therapy (90% CI: 51-77%); CR, PR, MR rates were 41%, 15% and 8% respectively. Two patients had serious adverse events (SAEs) with two episodes of venous thromboembolism (one deep vein thrombosis at platelet 97x10^9/L; one pulmonary embolism at platelet 240x10^9/L). 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 patient withdrew]. The median (Q1, Q3) dose eltrombopag at week 12 was 50 (50, 100)mg daily. The median (Q1, Q3) dose of steroid 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 day 42, PR and MR rates were 40% (90% CI 40-67%); CR, PR, MR rates were 28%, 21% and 5% respectively. Two patients had serious adverse events (SAEs) with two episodes of venous thromboembolism (one deep vein thrombosis at platelet 97x10^9/L; one pulmonary embolism at platelet 240x10^9/L).

There were no other adverse events or deaths.

**Summary/Conclusions:**
The majority of patients with ITP diagnosed for ≤6 months had a favourable overall response rate to eltrombopag and the drug was generally well tolerated. Longer-term follow up data (beyond 6 mos) will be presented at the meeting.

**E1432**

**A NOVEL RUNX1 MUTATION IN FAMILY WITH FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO ACUTE MYELOGENOUS LEUKAEMIA**

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**Background:**
MicroRNAs (miRNAs) are small noncoding RNAs involved in regulation of gene expression. Dysregulated expression of miRNAs has been associated with several autoimmune diseases. ITP is an autoimmune disease characterized by isolated thrombocytopenia and increased risk of bleeding. The development of autoantibodies against platelets and megakaryocytes results in increased platelet destruction and insufficient platelet production leading to pathophysiology of ITP. Platelets contain high levels of miRNAs and a substantial fraction of circulating miRNAs originates from platelets. Circulating miRNAs are stable and relatively easy to measure and considered as potential disease biomarkers. The role of miRNAs in the pathogenesis of ITP has not been well explored.

**Aims:** Determine the expression profile of circulating miRNAs in ITP patients in order to identify miRNAs that can be used as disease biomarkers and to explore the potential biological pathways that might be involved in the pathogenesis of ITP.

**Methods:** Exiqon Serum/plasma Focus microRNA PCR panel was used to determine the expression profile of 179 miRNAs in plasma acquired from 8 ITP patients with low platelet count and who failed to respond to various treatments for ITP, and from 8 age- and sex-matched controls. In addition, next generation sequencing (NGS) for miRNAs was performed in 2 pooled plasma samples (pool 1 from 4 ITP patients, and pool 2 from 4 matched controls), on the Illumina NextSeq 5500 system. Statistical analyses were performed with the GenEx software and SPSS. Pathway analysis was performed using DIANA-miPath v3.0 to explore the probable pathways involved in the pathogenesis of ITP.

**Results:**
Comparing the expression profiling from the PCR panel between ITP patients and matched controls, 81 circulating miRNAs were differentially expressed (p<0.05), of those 17 miRNAs had a high statistical significance (q<0.001). Four miRNAs were up-regulated and 13 miRNAs were down-regulated in ITP compared to controls. Interestingly, 15 of the 17 differentially expressed miRNAs from PCR panel were also differentially expressed in NGS. Using the 17 differentially expressed miRNAs in the miRPath analysis, we uncovered some immune system related pathways, including MYD88-independent toll-like receptor signaling pathway and TRIF-dependent toll-like receptor signaling pathway, as enriched pathways in target genes of miRNAs differentially expressed between ITP patients and controls.

**Summary/Conclusions:** We identified a large number of miRNAs that were differentially expressed in ITP patients compared to controls that might be associated with the pathogenesis of ITP. Pathways analysis uncovered some possible biological pathways that might be involved in the pathogenesis of ITP. Further validation of these miRNAs in a larger patient cohort and preferably in comparison to patients with other causes of thrombocytopenia such as aplastic anemia could explore the role of these miRNAs in the pathogenesis of ITP. Future studies of these miRNAs in relation to initiation of treatment with defined clinical outcomes as treatment response/ remission after initiation of treatment will clarify their potential as biomarkers for treatment response.
NORDIC COUNTRY PATIENT REGISTRY FOR IMMUNE THROMBOCYTOPENIA (NCPRTIP): A COHORT OF PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA IN DENMARK, SWEDEN, AND NORWAY

Background: Immune thrombocytopenia (ITP) is a rare disease characterized by isolated 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 NCPRTIP and the characteristics of patients enrolled.

Methods: Encompassing Denmark, Norway, and Sweden, the NCPRTIP started as a population-based post-authority safety study to assess the long-term safety of romiplostim in treating ITP. It includes patients with prevalent chronic ITP (cITP – ITP lasting >6 months) as of 04/01/2009 in 9 of 10 ITP 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 Danish national health registers and medical record review, the registry has rich clinical information for all enrolled ITP patients, as well as comorbidities (including scores according to the Charlson Comorbidity Index [CCI] – a validated tool developed to predict 1-year mortality), treatments, lab values (e.g., platelet counts), and complete follow-up for several clinical outcomes of interest (e.g., clinically significant bleeding, the need for rescue therapies, and thromboembolic/thrombotic events). Additionally, available bone marrow samples are restained and reexamined for reticulin and collagen content to assess Thiele’s myelofibrosis (MF) grading.

Results: The NCPRTIP 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 entry, 11% of the cohort had hypertension (9%), diabetes, and 18% had a history of hypertension. Currently, 718 bone marrow samples from 566 patients have been retrieved.

Summary/Conclusions: The NCPRTIP 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.

EPIEMIOLOGY OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ADULTS IN RUSSIAN FEDERATION (RESULTS OF REGISTRY OF NATIONAL HEMATOLOGIC ASSOCIATION)

Background: Immune thrombocytopenia (ITP) is a rare disease characterized by isolated 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.

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Methods: Encompassing Denmark, Norway, and Sweden, the NCPRTIP started as a population-based post-authority safety study to assess the long-term safety of romiplostim in treating ITP. It includes patients with prevalent chronic ITP (cITP – ITP lasting >6 months) as of 04/01/2009 in 9 of 10 ITP 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 Danish national health registers and medical record review, the registry has rich clinical information for all enrolled ITP patients, as well as comorbidities (including scores according to the Charlson Comorbidity Index [CCI] – a validated tool developed to predict 1-year mortality), treatments, lab values (e.g., platelet counts), and complete follow-up for several clinical outcomes of interest (e.g., clinically significant bleeding, the need for rescue therapies, and thromboembolic/thrombotic events). Additionally, available bone marrow samples are restained and reexamined for reticulin and collagen content to assess Thiele’s myelofibrosis (MF) grading.

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Summary/Conclusions: The NCPRTIP 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.

ELTROMBOPAG (EPAG) FOR THE TREATMENT OF PATIENTS AGED ≥65 YEARS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP): SAFETY AND EFFICACY RESULTS FROM THE EXTEND STUDY

Background: ITP is an acquired autoimmune disorder characterized by isolated platelet reduction, which is considered chronic when persisting for ≥12 months. Evidence suggests that age may influence both the hematologic 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 newly diagnosed and 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 newly diagnosed and persistent (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 continued to maximize platelet count response and optimize ITP dosing. Pts could remain on EPAG either for 2 yrs in countries where the NCIP persist for ≥12 months. Evidence suggests that age may influence both the hematologic 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 newly diagnosed and persistent (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.

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while not receiving rescue treatment, was 78 (range, 0–350) weeks. Incidence of bleeding symptoms (WHO grades 1–4) decreased from BL (86%) to 1 y (15%). AEs were reported in 47 (94%) pts, most frequently nasopharyngitis (n=13, 26%), constipation (n=12, 24%), fatigue (n=12, 24%), diarrea, arthralgia, urinary tract infection, cataract and cough (all n=11, 22%). Serious AEs occurred in 24 (48%) pts, most frequently >5% cataracts (n=7, 14%), pneumonia (n=6, 12%), and urinary tract infection (n=3, 6%). The most frequent AEs with suspected relationship to study drug were cataracts (n=8, 16%), headache, fatigue, and increased ALT, AST and bilirubin (all n=3, 6%).

## E1439

### CHILDHOOD IMMUNE THROMBOCYTOPENIA: A NATIONWIDE COHORT STUDY ON CONDITION MANAGEMENT AND OUTCOMES

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**Background:** Little is known about the management of pediatric ITP in real life, that is, routine clinical practice. Moreover, the predictive value of these factors upon disease outcome was explored individually and therefore the confounding effect of associated exposures remains unknown.

**Aims:** With this nationwide prospective cohort study, our objectives were to explore (1) the factors associated with treatment initiation (vs. watchful waiting) in children with primary immune thrombocytopenia (ITP) followed in routine clinical practice and (2) the predictors of chronicity at 12 months.

**Methods:** Between 2008 and 2013, 23 centers throughout France consecutively included 257 children aged 6 months to 18 years and diagnosed with primary ITP over a 5-year period. Data on ITP clinical features along with medical management were collected at baseline and 12 months. Multivariate logistic regressions were used to determine (1) and (2) as defined above, providing odds ratio (OR) with 95% confidence intervals (95% CI).

**Results:** 137 (53%) children were males, median age 4.6 years, median platelet count was 7×109/L, and 214 (81%) patients initiated medication. Factors independently associated with treatment initiation included platelet counts <10×109/L (p<0.0001) and mucocutaneous bleeding symptoms at baseline (p<0.001). At 12 months, data were available in 211 (82%) children, of whom 100 (74%) had recovered. Predictors of chronicity included female gender (OR=2.2; 95% CI=1.0–4.8), age ≥10 years (OR=2.6; 95% CI=1.1–6.0) and platelet counts ≥10×109/L (OR=3.2; 95% CI=1.5–6.9).

**Summary/Conclusions:** In routine clinical practice, the decision to apply a watchful-waiting strategy seems to be driven by platelet counts even in the presence of bleeding symptoms, resulting in treatment being initiated in more than 80% of the children surveyed. Overall, younger children with ITP showed good prognosis, with lower platelet counts and, to a lesser extent, male gender predicting more favorable outcomes.

## E1440

### SIROLIMUS FOR THE TREATMENT OF CHILDREN WITH IMMUNE THROMBOCYTOPENIA AND EVANS SYNDROME: A SINGLE CENTRE EXPERIENCE

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**Background:** The treatment of chronic relapsing immune thrombocytopenia purpura (ITP) is not well established due to the lack of evidence-based data, and is particularly challenging in children who are 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 ALPS1 and in very few patients with primary disease or secondary to ALPS-like syndromes.2

**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 primary additional criterion for ALPS. Complete response (CR) and partial response (PR) were defined as a platelet count >100×109/L and >30×109/L and at least 2 fold increase of the baseline count, respectively.

**Results:** 23 children aged 0-12 yrs (median 6) with primary ITP (7) or secondary to 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 PI3KCD, CTLA4, TACI, and CARD 11 gene. All patients, but one treated in first-line, received Sirolimus. Seven patients were treated as second (4), third (14) or fourth (4) line treatment, respectively. Prevalent side effects were limited in 67 (86%) cases. Children with mono-linear ITP achieved a response in 11/16 (74%) and at least 2 fold increase of the baseline count, respectively.

**Summary/Conclusions:** To the best of our knowledge this is the largest cohort of patients with ITP or ES other than ALPS -treated with Sirolimus, that showed to be safe and effective in most cases, including patients who previously failed
MMF treatment. Therefore, it can be considered as an alternative therapeutic option in the setting of ITP non only for patients with an underlying diagnosis of ALPS but also for the ones with primitive disease or with an ALPS-like disorder.

References

RESULTS
The mean age of patients and control was 29.5±2.8 yrs; 13.86 yrs and 27.90± 8.89 yrs respectively. Male/Female ratio in patients and control was 1:2. Under additive model, the heterozygous genotype (AG) of the FcγRIIA 131 H/R (A>G) polymorphism shows the significant association with ITP, (Odds Ratio 2.41 (95% CI, Lower - 1.19 Upper 4.90 P-value 0.0149) whereas the homozygous mutant genotype (GG) had no significant association with ITP (Odds Ratio 2.47 (95% CI, Lower - 0.63 Upper 9.72 with P-value 0.2976). Under dominant model, the Odds Ratio was 2.42 (95% CI, Lower - 0.34 Upper 9.94) with the significant P-value 0.0167. Mutant allele (G) frequency was 37.85% in patients and 25.71% in controls (Odds ratio 1.76 1.05-2.93 with the p-value 0.0297).

Summary/Conclusions: The study shows the association of heterozygous genotype (AG) of FcγRIIA 131 H/R (A>G) with ITP. The dominant model also shows significant association with ITP. We conclude that mutant allele (G) in FcγRIIA 131 H/R (A>G) gene polymorphism may have impact on susceptibility to ITP.

E1441
ASSESSMENT OF ROMIPLOSTIM SELF-ADMINISTRATION BY PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA AND CAREGIVERS FOLLOWING RECEIPT OF HOME ADMINISTRATION TRAINING (HAT) MATERIALS:A PROSPECTIVE SECTIONAL 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 thromboepoetin- receptor agonist which is approved in the European Union (EU) to treat chronic immune thrombocytopenic purpura (ITP) refractory to other treatments.

Aims: To estimate the proportion of adult patients and caregivers who administer romiplostim 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 nursing intervention to read the correct dose from the vial due to poor eyesight, and 1 caregiver needed guidance with syringe and vial connection. Further follow-up data was available for only 2 of these 5 patients/caregivers; they both administered romiplostim correctly at a voluntary subsequent visit.

Summary/Conclusions: Given that this study was conducted on a convenience instead of random sample of patients, generalizability of the results may be limited. Direct observation can be susceptible to observation bias and to the Hawthorne effect with the patients/caregivers acting differently when observed. Nonetheless, the success of most patients and caregivers in correctly administering romiplostim after HAT pack training suggests that self-administration of romiplostim is a feasible option for suitable romiplostim-treated ITP patients.

E1442
FCγIIA 131 H/R (A>G) RECEPTOR GENE POLYMORPHISM IN PATIENTS OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)
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Background: Primary Immune Thrombocytopenia (ITP) is an autoimmune hematologic disorder characterized by isolated thrombocytopenia (<100,000/mm3) in the absence of other causes or disorders that may be associated with thrombocytopenia. The predominant mechanism is enhanced peripheral destruction of autoantibody coated platelets through binding of Fc portion of antibody with the Fcγ receptors on cells of reticuloendothelial system mainly monocytes/macrophages.

Aims: This study was aimed to investigate the association of polymorphisms in FCγIIA 131 H/R (A>G) with Primary Immune thrombocytopenia (ITP).

Methods: Genotyping for the FCγIIA 131 H/R (A>G) was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) in 70 ITP patients and 70 healthy controls.

Figure 1.

Results: A total of 45 pts (M: 21) were eligible for analysis; median age at treatment was 60 yrs (range 18-87) and median time between diagnosis and treatment 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 80mg in ITP along with 1st Dxm course were required in 11/45 pts. In between courses, no bleeding complications were observed and no emergency therapies were required. Short-term response was achieved in 39/45 (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 pvl count at last
follow-up was 102±10.9 days (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 day clinical practice. The role of a reduced-dose schedule needs to be explored in a larger cohort of pts. Treatment of newly diagnosed ITP pts - i.e. within 3 mos of diagnosis (Rodeghiero Blood 2007) - seems to lead to longer RFS.

E1444
EFFECT OF OSELTAMIVIR TREATMENT ON PLATELET COUNTS
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Background: As platelets lose sialic acid during aging and circulation, they are cleared by the hepatic Ashwell-Morell receptor (AMR) (1). A recent study suggests that inhibition of sialidase by oseltamivir, a commonly administered anti-influenza medication that inhibits viral sialidase, could associate with an increase in platelet counts (2).

Aims: The aim of this study was to analyze the effect of oseltamivir treatment in platelet counts.

Methods: We performed a retrospective single-center study. From November 2009 until March 2015, a total of 168 patients from our Hematology Unit were prescribed oseltamivir due to clinical suspicion of influenza. A total of 120 patients were excluded because they had received myelotoxic chemotherapy within 30 days (n=82) or platelet count was not available before treatment (n=38). The direct immunofluorescent antigen test was carried out with 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-genic stem cell and transplant recipients). We observed a significant increase in the mean platelet count after treatment with oseltamivir (170±95 x10⁹/L vs 192±103 x10⁹/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 x10⁹/L vs 182±91 x10⁹/L).

Table 1.

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 TTP. Using a recently developed test for ADAMTS13, we showed that 58% of patients had a reduced level of ADAMTS13 in TTP. There is scant evidence on the best investigations for patients suspected of being at risk of cardiac complications with no evidence on the clinical utility of cardiac magnetic resonance imaging (MRI) in acute TTP episodes.

Aims: A retrospective review evaluating the value of cardiac MRI scanning in TTP. Medical records of patients under treatment for cardiologic symptoms between November 2008 and November 2014 whilst being treated for an acute episode of immune mediated TTP. All patients had troponin-I 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-I 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 atrial fibrillation. 88% of patients had at least one abnormal finding in their transthoracic echocardiogram. LGE seen in combination with regional wall motion abnormalities (RWMA) suggests irreversible myocardial dysfunction. No patient was found to have RWMA on transthoracic echocardiogram but this was seen in five patients on cardiac MRI imaging, all of whom also had LGE. The mean troponin-I level was increased in those with an abnormal cardiac MRI (normal MRI 100ng/ml, abnormal MRI 165ng/ml, p=0.9), nor was there a significant difference in median age (49 vs 49), symptom duration (abnormal MRI 7 days, normal 5 days, p=0.39) or presenting anti-ADAMTS13 antibody level (abnormal MRI 41%, normal MRI 40%, p=0.66).

Summary/Conclusions: Cardiac MRI scanning in TTP is a sensitive tool for detecting ischemic cardiac changes that would otherwise be missed by transthoracic echocardiogram. Mid-Apical late gadolinium enhancement appears to be a characteristic finding in TTP. These findings help increase the understanding of the pathophysiology behind the TTP disease process.

E1446
THE FREQUENCY AND CLINICAL SIGNIFICANCE OF MEFV GENE MUTATIONS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA
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Background: Immune thrombocytopenia (ITP) is an immune-mediated acquired disease characterized by transient or persistent decrease of the platelet counts. MFEV gene mutations are responsible for Familial Mediterranean Fever (FMF) a hereditary autoinflammatory disease characterized by recurrent febrile inflammatory attacks of serosal and synovial membranes. MFEV gene’s protein product, pyrin or marenostrin, play an essential role in recurrent febrile inflammatory attacks of serosal and synovial membranes. Mutations in the MEFV gene cause an increase in Th17 numbers. Th17 cells may have a key role in neutrophil activation and autoimmune diseases. Recently, mutations in the MEFV gene were found in a subset of ITP patients.

Aims: We aimed to reveal the frequency and clinical significance of MEFV mutations in a population presenting with 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
Summary/Conclusions: Our findings indicate that PD-1 gene polymorphisms contribute to the susceptibility of cITP, and PD-1 low producer genotype affects the severity of cITP. In addition, CTLA-4 high producer genotypes suggest the good clinical features and a little requirement of treatment in patients with cITP.

E1448
IS THE SPLENECTOMY OUTCOME PREDICTABLE IN PATIENTS WITH ITP?
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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 for optimizing treatment outcomes. Accordingly, it is important to identify pre- or post-operative parameters that are able to predict the response to splenectomy.

Aims: To identify the pre- and post-operative 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 0-10; median platelet count before splenectomy 23.6%; p=0.043, 0.003 and 0.018, respectively). On the other hand, CTLA-4 -1577 AA genotypes was significantly associated with higher minimum platelet count than AG & GG genotype (22.5 vs 14.0 ×109/L, p=0.048).

PD-1 AND CTLA-4 POLYMORPHISMS AFFECT THE SUSCEPTIBILITY AND CLINICAL FEATURES OF CHRONIC IMMUNE THROMBOCYTOPENIA
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Background: The programmed death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) play a central role in immune checkpoint pathways. The PD-1 negatively regulates self-reactive T and B cells in peripheral immune tolerance. The CTLA-4 antagonizes the binding of CD28 to its ligands including CD80 and CD86, and inhibits T cell activation. Previous studies have shown the lower expression of serum soluble PD-1 and CTLA-4 mRNA in patients with chronic immune thrombocytopenia (cITP) than healthy individuals. Single nucleotide polymorphisms (SNPs) of PD-1 and CTLA-4 have been reported to be associated with susceptibility of some autoimmune diseases; however, the possible association between these immune checkpoint SNPs and cITP risk remain controversial and obscure.

Aims: In order to explore the role of PD-1 and CTLA-4 in the pathogenesis of cITP, we investigated the impact of PD-1 and CTLA-4 SNPs on the susceptibility and clinical features of adult cITP.

Methods: We extracted the genomic DNA from 141 cITP patients and 223 healthy controls, and determined, 3 PD-1 SNPs (-606G/A, -7290G/C, 2A15V) and 4 CTLA-4 SNPs (-1722T/C, -1577G/A, +49A/G, +6230G/A) by using the polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP) method. The severity of bleeding tendency and thrombocytopenia was assessed according to the previously described criteria by Han JJ. The risk criteria, ”corticosteroid dependence”, severe CTP, and refractory cTP were used to define the criteria for the response of cITP to corticosteroid therapy.

Results: The minimum platelet count of all clinical course ranged from 0 to 98×109/L with a median count of 13×109/L. Eighty-six patients (61.0%) had bleeding tendency and 24 patients (17.0%) had severe thrombocytopenia (<10×109/L). Eighty-six patients (61.0%) received the treatment with corticosteroids, and 11 patients (8.2%) were corticosteroid-dependent. Twenty-four patients (17.0%) were responsive to healthy controls, the higher frequency of PD-1 -7290 TT genotype (low producer) was observed in cITP patients (12.8% vs 4.5%, p=0.004). There were no significant differences in CTLA-4 SNPs between cITP patients and healthy controls. In cITP patients, PD-1 -7290 TT genotypes (low producer) was significantly associated with high frequency of treated patients, treated patients with corticosteroid, and corticosteroid-dependent patients compared with CC & CT genotype (high producer) (94.4% vs 71.5%, 94.4% vs 57.7% and 52.9% vs 23.8%; p=0.043, 0.003 and 0.018, respectively). On the other hand, CTLA-4 +49 AA genotype (high producer) was significantly associated with low bleeding tendency and thrombocytopenia (AG & GG genotype (low producer) (27.3% vs 63.8%, p=0.017). CTLA-4 -1577 AA genotypes (high producer) was significantly associated with low bleeding tendency and steroid treatment than AG & GG genotype (low producer) (26.4% vs 65.6%, p=0.010). CTLA-4 -6230 AA genotypes (high producer) was significantly associated with higher minimum platelet count than AG & GG genotype (22.5 vs 14.0 ×109/L, p=0.048).

E1449
FINAL RESULTS FROM AN OBSERVATIONAL STUDY (PLATEAU) OF ADULT PATIENTS TREATED WITH ROMIPOSTIM FOR PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ROUTINE CLINICAL PRACTICE IN GERMANY
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Background: In the European Union, the thrombopoietin-receptor agonist romipos tin (Nplate®) is recommended since January 2016 for treatment of ITP in patients who are refractory to other treatments (e.g. corticoids, immunoglobulins).

Aims: The aim of this study was to assess the use of romipos tin in clinical practice in Germany.

Methods: This multicentre, prospective and retrospective observational study of patients with chronic immune thrombocytopenia (romipos tin) enrolled ITP patients >18 years who received at least one dose of romipos tin in routine clinical practice, with an observation period of 2 years following romipos tin initiation. Endpoints included patient demographics, romipos tin use, platelet counts, adverse
drug reactions (ADRs), and other clinically relevant parameters. We report results from 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 periods from randomization to 15.5 months. Data were collected from 102 patients (61%). The median platelet count rose sharply from (29.0 × 10^9/L) to two weeks of treatment (62.5 × 10^9/L). From week 3 to 6 months, the safety platelet data maintained a range between 50 and 101 × 10^9/L and 145.5 × 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 57 patients (27.0%). The most frequent ADRs were gastrointestinal (10.2%) and psychiatric (11.7%) ADRs, followed by constitutional symptoms (10.9%). Adverse drug reactions pertaining to blood/bone marrow affected 2.9% of patients (vascular/thrombotic events, bone marrow fibrosis), whereas bleeding as an ADR was seen in 0.7% of patients. The exposure-adjusted rate of bleeding events (grade 3 or 4) per 100 patient-years in the FAS was 7.2 before treatment vs 4.0 after starting the treatment. The rate of ITP-related hospitalization per 100 patient-years decreased from 23.3 before the start of therapy to 15.5 since the start of 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 100 x 10^9/L over time, regardless of the splenectomy status of the patients; most of them were non-splenectomized. The product was well tolerated and achieved a decrease in the rate of ITP-related hospitalization.

E1450
THE CLINICAL UTILITY OF NEUropsychology Testing in IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: It is well recognized that neurological manifestations are common in thrombotic Thrombocytopenic Purpura (TTP) however research into the neuropsychological impact of the disease is lacking despite evidence suggesting patients who experience critical illnesses are at high risk for long-term cognitive impairment.

Aims: To review the clinical utility of neuropsychology testing in thrombotic thrombocytopenic purpura.

Methods: Between 2010 and 2015, all patients within a single tertiary haematology center with a confirmed diagnosis of TTP were reviewed as outpatients for neuropsychological assessment. The Wechsler Adult Intelligence Scale III (WAIS-III) IQ test was used to assess factors including verbal IQ and performance IQs.

Results: 18 patients were included. 89% were female with a median age of 51 (16-67 years). 56% were Caucasian, 33% Afro-Caribbean and 11% of South Asian ethnic origin. 33% had experienced TIA or stroke-like symptoms during the acute phase. Only 29% had no neurological symptoms during their initial presentation. The most common symptom leading to neuropsychology review was problems with concentration, experienced by 89% of patients. 44% had problems with memory, 39% felt depressed and 33% had anxiety issues. The median time from acute TTP episode to neuropsychology review was 29 months (range 3-90 months). 50% had signs of sub-acute infarction on imaging and two patients scans showed both mature infarcts and microhaemorrhages. The median scores for both verbal and performance IQs were reduced compared to normal (100, range 90-110). The median verbal IQ was 87 (range: 65-122) and the median performance IQ was 93 (range: 80-100). Taking all aspects of the WAIS-III into consideration, one patient had a normal assessment. 50% (n=9) were found to have mild cognitive impairment, 33% (n=6) mild-moderate impairment and 11% (n=2) significant impairment. The two cases with significant impairment had a widespread pattern of dysfunction whilst in the other cases the most common deficits were sub-cortical/cognitive.

Summary/Conclusions: Persisting psychological symptoms after an acute TTP episode are highly suggestive of underlying cognitive impairment as a result of cerebral sub-acute infarction or microhaemorrhages.

E1451
FIVE NEW CASES OF HERMANSKY-PUDLAK SYNDROME: IDENTIFICATION OF NOVEL GENETIC VARIANTS IN HPS4 AND HPS3 ASSOCIATED TO RELEVANT CLINICAL COMPLICATIONS

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Background: Hermansky-Pudlak syndrome (HPS) is an inherited platelet disorder characterized by bleeding diathesis, oculo cutaneous albinism and some-times serious clinical complications. Heterogeneous clinical symptoms and a large number of possible genetic culprits (9 HPS genes, >110 exons) complicate unequivocal HPS diagnosis.

Aims: To assess the clinical and platelet phenotype in five patients with HPS suspicion and to identify their genetic defects (through high throughput sequencing).

Methods: We studied 5 patients from 3 families (2 Spanish, 1 Turkish) presenting with oculocutaneous albinism. Clinical records were reviewed and bleeding scored using ISTH-BAT. Platelet phenotyping (only Spanish patients) included: platelet aggregation, GP2 expression and granule secretion. 14C-serotonin uptake and whole mount electron microscopy. Patients DNAs were analyzed using HTS using a 71 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 46yr 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 colostomy (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.

Table 1.

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Race</th>
<th>Ethnicity</th>
<th>Ocular Manifestations</th>
<th>Cutaneous Manifestations</th>
<th>GI Bleeding Past History</th>
<th>Liver Enzyme Abnormalities</th>
<th>Platelet Aggregation</th>
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<tr>
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<td>Talcott</td>
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<td>No</td>
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</tr>
<tr>
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<td>M</td>
<td>C</td>
<td>33%</td>
<td>Talcott</td>
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</tr>
<tr>
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<td>Talcott</td>
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<td>No</td>
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<td>Normal</td>
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</tr>
<tr>
<td>P1</td>
<td>M</td>
<td>T</td>
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<td>Talcott</td>
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<tr>
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<td>T</td>
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<tr>
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<td>F</td>
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</tbody>
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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.

E1452
CHARACTERIZATION OF PLATELET ACTIVATION MARKERS IN EARLY ONSET PREECLAMPSIA

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Background: Preeclampsia is a serious pregnancy complication with potentially life-threatening consequences for both mother and baby, diagnosed when new onset hypertension and proteinuria develops after 20 weeks gestation. Early onset preeclampsia (EOP; onset <34 gestational weeks), is associated with higher maternal and fetal risks than late onset preeclampsia. At the extreme end of the severity spectrum, HELLP syndrome is characterised by...
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) and compare it with healthy controls.

Methods: Plasma samples from 191 healthy controls and 64 patients with EOP were collected. Platelet activation was assessed using ELISA and flow cytometry. Platelet microparticles were measured by flow cytometry. Platelet activation biomarker levels were adjusted by platelet count and expressed as %/10^8 platelets/ml. All data was analysed using GraphPad Prism 7. Parameters were reported as means±SEM.

Results: Plasma samples from 19 individual patients were included. Patients with HELLP syndrome demonstrated significantly greater numbers of CD42a+ microparticles when corrected for platelet count compared with those without HELLP syndrome (598x10^3±203x10^3 versus 297x10^3±37x10^3, p=0.04). Similarly, patients with HELLP syndrome demonstrated increased levels of sGPVI than those without HELLP; corrected for platelet count (2.57±0.9667 versus 1.22±0.124 ng/ml, p=0.0334). There was no difference in NAP-2 or PF4 levels between those with HELLP and those without HELLP, nor between severe and moderate pre-eclampsia patients. Severe pre-eclampsia patients in this cohort had a D-dimer level of 3.7±10:742 µg/ml compared with non-severe patients 1.85±0.350 µg/ml (t=2.403, p=0.0337). The correlation between sGPVI levels and D-dimer levels (Spearmann Rank correlation coefficient, r=-0.532, p=0.04).

Discussion: Our data suggests that platelet activation is associated with HELLP syndrome. The increased levels of platelet microparticles and sGPVI in patients with HELLP syndrome suggests a role for 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.

E1453 PRIMARY ITP IN ADULTS TREATED WITH ELTROMBOPAG: A RETROSPECTIVE STUDY USING DATA FROM THE UNITED KINGDOM ADULT IMMUNE THROMBOCYTOPENIA REGISTRY.

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3ADULT IMMUNE THROMBOCYTOPENIA REGISTRY

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 therapy has remained unchanged for decades and until recently, second-line therapy has been unsatisfactory, using empirical treatments. The recently approved thrombopoietin receptor agonists 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%).

Summary/Conclusions: This is the first UK registry-based study of adult ITP patients treated with eltrombopag. Although limited in size, these data suggest several important points: the majority of patients were older, and the median time from ITP diagnosis to eltrombopag initiation was 1.6 years. As clinicians become more familiar with its use, a greater proportion of patients are likely to receive eltrombopag as a second line therapy.
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 Rodeghiero 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%) thromboembolic events. The most frequent venous TEE were pulmonary embolism, deep vein thrombosis, and superficial vein thrombosis; arterial TEE were cerebrovascular insults, myocardial infarction and peripheral artery thrombosis. At time of TEE, 43% of patients were on treatment with corticosteroids, 14% with thrombopoietin receptor agonists (TPO-ra) and 18% were off-treatment. In the univariate analysis, older age at diagnosis (<50 years, P=0.015), longer interval since ITP diagnosis (P=0.009), ≥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; P=0.041) revealed to be significant. The multivariate analysis model (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%).

Summary/Conclusions: Adult ITP patients are at risk for thromboembolic events. Patients older than 50 years, having a persistent/chronic form of the disease, requiring two or more lines to treat the ITP, previous splenectomy, and smokers were more likely to develop TEE. The knowledge about the risk of thromboembolic events in adult ITP patients could have an impact on management attitude for patients at risk.

OSELTAMIVIR FOR THE TREATMENT OF ITP PATIENTS NOT RESPONDING TO CONVENTIONAL TREATMENT: BIOLOGICAL CHARACTERIZATION AND CLINICAL RESPONSES

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Background: Oseltamivir phosphate, a drug that conventionally serves as an antiviral sialidase inhibitor classically prescribed for the treatment of patients with influenza, has shown to induce an increase in platelet counts in 2 patients with primary immune thrombocytopenia (ITP) (1,2). A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance with primary immune thrombocytopenia (ITP) (1.2). A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance with influenza, has shown to induce an increase in platelet counts in 2 patients with primary immune thrombocytopenia (ITP) (1,2). A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance with influenza, has shown to induce an increase in platelet counts in 2 patients with primary immune thrombocytopenia (ITP) (1,2). A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance with influenza, has shown to induce an increase in platelet counts in 2 patients with primary immune thrombocytopenia (ITP) (1,2).

Aims: To analyze the biological features and clinical responses following oseltamivir treatment in patients that are non-responders to conventional treatments.

Table 1.

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 fluorescein-conjugated Ricinus 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, FXII) and HPLC (transferrin). Platelet autoantibody 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 75mg twice 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-
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 amount of time spent without economic activity and travel costs add up during the course of therapy. From the health-care provider the process of safely administering bortezomib has significant resource implications beyond those of drug procurement.

Aims: We set up a time-in-motion study to evaluate the costs to health care provider and patients during bortezomib therapy to estimate the ‘real-world’ cost of delivering bortezomib therapy.

Methods: Retrospective data collection was undertaken, using electronic prescribing records for patients treated between July 2014 -August 2016. Travel distance and time was estimated using Google maps and costed using HMRC mileage (an approved costing of mileage used for taxation purposes). The NHS schedule of service costs was used to estimate the cost of bortezomib administration. Cost of delivery of Bortezomib for healthcare providers is a sum of these individual costs.

Results: We identified 127 patients who incurred a total of 2,134 visits whilst receiving Bortezomib therapy at the Churchill Hospital in Oxford during this 2 year period. Median age was 70 years-old (yo) (39-95); Male 74 patients (58%) 53 patients (42%). We restricted the analysis to 110 patients who started and completed therapy during the study period. Median number of patient visits was 16 (range 11-52). The median travel distance (return journey) for each patient was 33 miles (53 km) (range: 1.2-224 mi; 1.9-360 km). Median travel time was 90 min (range: 8-300 min). The range travel cost per patient was £8.35-£13.20. Twenty-seven patients (21%) required use of specialist hospital transport services, which resulted in 295 transport-episodes (14%) in total. In order to assess the time spent in the day therapy unit, a subgroup of 589 patient-episodes were analysed to assess time from arrival to administration of Bortezomib: the median time from patient registration to bortezomib administration was 63min (range: 5-433min). Pharmacy cost for preparation of Bortezomib was £50 per dose. The cost of delivery of bortezomib (not including cost of drug) was £1,160 per cycle, which equated to a total median cost of £4,640 per patient (range: £290-£15,080). Drug procurement costs for Bortezomib is estimated at an additional £12,261 per course of therapy (BNF 2016). Delivery costs therefore added an additional 38% to the procurement costs.

Summary/Conclusions: We provide the first time-in-motion data on myeloma patients treated with Bortezomib. The ‘real-world’ cost of delivering therapy is 37% higher than the drug-costs alone. In addition the impact on patients is substantial: over a two year period 127 patients required 2,134 visits with a median time in the day unit of 63 minutes and a median travel time of 90 minutes per visit. Our data highlights the burden of both time and economic costs to patients during therapy. Novel oral proteasome inhibitors offer the potential to reduce this resource impact in the future. This data could be used by health care providers and reimbursing agents for economic modeling of the potential benefits of oral proteasome inhibitors.

E1458
HOSPITAL CARE AT HOME ADMINISTRATION OF SUBCUTANEOUS AZACITIDINE IS FEASIBLE AND PREFERRED BY PATIENTS COMPARED TO HOSPITAL ADMINISTRATION: A FRENCH REGIONAL HEMATOLOGY NETWORK EXPERIENCE
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Background: In France, azacitidine (AZA) is indicated for the treatment of adult patients affected by Myelodysplastic Syndrome with intermediate-2 or high risk according to the International Prognostic Scoring System (IPSS), Chronic Myelomonocytic Leukemia (CMLL) with 10-29% medullary blasts and Acute Myeloblastic Leukemia (AML) with 20-30% blasts. It’s also a drug treatment of adult AML patients over 65 years with >30% of medullary blasts. Azacitidine is an antimitotic agent, administrated once a day for 7 days of each 28-day cycle. It is used mainly for patients who could not tolerate effective, treatment cycles require frequent hospital visits which could decrease patient comfort and increase medical personnel workload. Limousin is a region with the oldest population of France and with a very low population density. There is one university hospital and two local state-run hospitals each with a hematology department. In 2009, HEMATO for the Limousin hematology network, set up a protocol called ESCADHEM (externalization and securitization of injectable chemotherapy at home for malignant hematological diseases) that facilitates chemotherapy administration via local hospital at Home (HaH) establishments, which is an alternative to conventional hospitalization in France (www.frenchad.fr). This study aimed to minimize the frequent hospital visit of the patients that these treatments require. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated preparation unit for cancer treatments. From 2009 to 2015, a total of 11,367 infusions were administrated at home for 464 pts. In 2016, we demonstrated the feasibility of ESCADHEM and the medico-economic interest of such care with infusions were administrated at home for 169 pts with AML/MDS received AZA therapy. From 2009 to 2015, 169 patients were treated with AZA by a combination of oral and subcutaneous AZA, is in process and results will be presented at the EHA meeting.

Aims: Our work aimed to demonstrate that HaH administration of AZA is feasible and well preferred by patients compared to hospital administration.

Methods: Chemotherapy at home obeys to strict rules. The first chemotherapy cycle (C1) and the first infusion (D1) of subsequent cycles were administrated at the outpatient care unit. The following injections were administrated at the patient’s home and carried out by HaH, according to a predefined procedures (Fig 1) to comply with safety rules essential to the protection of the professional, the patient, the entourage and the environment. Subcutaneous AZA injections were administrated at 101 (60%) pts with AML/MDS patients and 81 (50%) pts with AML. Patients received a median number of 5 cycles (1-41) and 26 injections of AZA (1-244) at home. The total duration of HaH management lasted from less than 1 day to more than 3.4 years with a mean of 6.3 months. During the period of HaH administration of AZA, 101 (60%) pts had to return to the hospital unit for a non programmed rehospitalization: 90% of the time the patient needed a transfusion, 4% because of infection and 6% for other reasons.

Results: From 2009 to 2015, a total of 6369 subcutaneous injections of AZA were administrated at home for 169 pts with AML/MDS received AZA therapy. Among all pts, 110 were males and 59 females with a median age of 75 years (range 41-92) there are 88 (52%) MDS patients and 81 (48%) pts with AML. Patients received a median number of 5 cycles (1-41) and 26 injections of AZA (1-244) at home. The total duration of HaH management lasted from less than 1 day to more than 3.4 years with a mean of 6.3 months. During the period of HaH administration of AZA, 101 (60%) pts had to return to the hospital unit for a non programmed rehospitalization: 90% of the time the patient needed a transfusion, 4% because of infection and 6% for other reasons.

Summary/Conclusions:Administration of oral analgesia and anxiolysis is a safe and feasible option to be used in outpatient setting: sedo-analgesia is very effective in reducing pain during the biopsy and diminishes the anticipatory anxiety related to a painful procedure. Patients should have the possibility to choose between local anesthesia alone or sedo-analgesia plus local anesthesia. The results of the satisfaction survey, focused on pts treated with subcutaneous AZA, is in process and results will be presented at the EHA meeting.

Table 1.

Background: Bone marrow aspiration and biopsy (BMAB) is a painful procedure, and the commonly adopted local infiltration anesthesia (LIA) with lidocaine is unable to relieve the pain during the most uncomfortable phases, or the anticipatory anxiety related to pain recalling thereafter. As there are no formal guidelines for adding a sedoanalgesic premedication before beginning the BMAB, many combinations have been adopted by several authors.

Methods: Patients were randomly assigned into two arms for receiving either sedoanalgesic placebo plus LIA (standard group, 48.6%) or oral fentanyl citrate 200 mcg plus oral midazolam 5mg in addition to LIA (combo-group, 51.4%) during BMAB. Pre-procedural anxiety and procedural pain were assessed according to the Numered Rating Scale (NRS: 0-10), dividing the time of the procedure into five intervals (T0, T1, T2a, T2b, and T3) and evaluating discomfort grade during each moment of procedure in both groups. Cognitive function was measured before and 30 minutes after the procedure. Possible side effects were recorded, as well as the adequacy of tissue samples harvested. A telephone interview was performed 24 hours later: A total number of one-hundred-sixteen (n=116, Table 1) were enrolled in the study. Nine (n=9) patients did not meet inclusion criteria and were excluded. Fifty-two (n=52) patients were randomized and assigned to standard group and fifty-five (n=55) to combo group.

Results: At T2b (corresponding to the biopsy time and time after the biopsy, respectively) there was a significantly lower (p < 0.05) perception of pain in the patients who received sedo-analgesia (combo-group) compared to those who did not (standard group). Moreover, 100% of the patients in combo group who had previously undergone this procedure without premedication, reported that they would prefer sedoanalgesia for the subsequent procedures, thus showing the effectiveness of this combination also in relieving anticipatory anxiety. Finally, the histological specimen was found to be high in quality, as defined by standards.

Table 1.
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 five years) clinical and economic outcomes for patients with AA receiving either ATGAM or r-ATG as first-line IST treatment. The follow-up key assumptions were included in the model: responders who relapse are assumed to be re-treated with no expected change in survival. Patients that 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 first-line. Patients who continue to not respond receive standard supportive care with a significant decrease in expected survival. Efficacy data for ATGAM and r-ATG were obtained from published literature. Adverse events were not included due to lack of evidence of any difference between the two comparators. Medication, administration, and disease management costs were obtained from published literature, publicly available sources and clinical expert opinion. As resource utilization for disease management changes over time and differs considerably between responders and non-responders, three distinct phases have been included in the model: short-term (first 6 months post-IST administration), medium-term (6-12 months) and long-term (greater than 1 year), for patients in either of the response categories.

Results: Response to treatment was calculated to be seen in 67% of ATGAM patients’ vs 35% in r-ATG (accounting for mortality). Over 5 years, the model estimated that patients gained 4.15 life-years (3.28 quality-adjusted) on ATGAM vs 3.52 (2.56) on r-ATG. Short-term disease management costs were estimated to be SEK 800,144 (€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 armamentary 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 tx. Data collected pertained to pt characteristics, tx patterns, duration of tx and outcomes (including time to progression (TTP) and best response achieved (IMWG updated criteria), HCRU in terms of hospitalizations, additional supportive drugs prescribed and healthcare professional (HCP) visits. Pt records included in this interim analysis were completed by Feb 17th 2017, with data collection continuing in all countries.

Results: 214 record forms have been reviewed to date. Pts’ mean age at diagnosis was 59 (±7.8 SD) years; 43% female and 57% male. Mean duration from diagnosis to receiving an ASCT was 9.6 months (±13.3 SD). Of the pts included in the study, 62%, 28% and 8% had received 1st, 2nd and 3rd line tx respectively. In the 1L setting, 72% of pts did not receive any drug therapy post 1L tx, 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 (11%). 22.2% of 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. The period during which pts did not receive any drug therapy post 2L tx was 22.2 months (±11.1 SD) for pts not receiving maintenance and 33.0 months (±8.1 SD) for pts receiving maintenance. Overall 43% of pts achieved a sCR and CR, 51% achieved a VGPR and PR. The period during which pts did not receive any drug therapy post 2L tx was 22.2 months (±11.1 SD) for pts not receiving maintenance and 33.0 months (±8.1 SD) for pts receiving maintenance. Overall 43% of pts achieved a sCR and CR, 51% achieved a VGPR and PR.

Summary/Conclusions: The sample is reflective of the pt demographics data reported in Raab et al. 2016. Furthermore, the TTP for pts not receiving any active ongoing tx post ASCT in this real-world study is comparable to findings in the literature. Although 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 chemotherapeutic regimen for the treatment of children, adolescents, and adults with acute lymphoblastic leukaemia (ALL). Compared to native asparaginase (native ASP), pegaspargase (PEG-ASP) has a longer half-life, can be given less frequently, and is less immunogenic, which leads to fewer hypersensitivity reactions. In the UK, patients with newly diagnosed ALL are treated with PEG-ASP followed by Erwinia-derived asparaginase (ERW-ASP) in cases of hypersensitivity, based on the UKALL protocols. Although native ASP is no longer used as the first choice of asparaginase therapy, it was the standard of care before PEG-ASP was available. A cost-utility analysis (CUA) was conducted to evaluate overall cost-effectiveness of PEG-ASP in comparison to native ASP when utilized as part of antineoplastic combination therapy for treating newly diagnosed ALL in children, young people, and adults.

Aims: To evaluate the cost-effectiveness of a treatment strategy including PEG-ASP in a multi-agent in patients with newly diagnosed ALL compared to regimens that include native ASP.

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 ineligible 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 U/m2 dosing (per UKALL protocols) used for treatment with PEG-ASP, the 2,000 U/m2 dosing (per Seppi protocol) of PEG-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.

Table 1.

<table>
<thead>
<tr>
<th>Technologies</th>
<th>Total Cost (€)</th>
<th>Incremental ICER (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-ASP &gt; ERW-ASP</td>
<td>€7,871</td>
<td></td>
</tr>
<tr>
<td>ERW-ASP &gt; PEG-ASP</td>
<td>€5,324</td>
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* Dominates indicates more cost and less benefit compared to the alternative strategy
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 VENETOCLAX ON THE QUALITY OF LIFE OF PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS OF A PHASE 2, OPEN-LABEL STUDY OF VENETOCLAX (ABT-199/ GDC-0198) MONOTHERAPY

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Background: Chronic lymphocytic leukemia (CLL) is associated with reduced health-related quality of life (HRQoL), with progressive severe fatigue being a particularly relevant burden. Disease-related symptoms, toxic effects of therapy, and the awareness of living with an incurable disease can have a profound impact on HRQoL. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.

Aims: To assess whether Venetoclax has a sustained impact on health related quality of life among patients with relapsed/refractory CLL based on a second interim analysis (first interim results through week 24) of patients treated with VEN monotherapy.

Methods: Patients ≥18 years of age with R/R CLL received VEN monotherapy until disease progression, unacceptable side effects, or discontinuation for any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16, 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 MID acceptance for both measures.

Results: Clinically meaningful improvements from BL were observed early and were sustained through week 96 in VEN treated patients in the EORTC-QLC-C30 global health status and the role, social, and emotional functioning scales. Improvements in VEN treated patients in EORTC-QLC-CLL16 based on a second interim analysis (first interim results through week 24) of patients treated with VEN monotherapy.

Table 1. Table 1.}

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.

E1467 WHICH HEMATOLOGICAL CONDITIONS CAN THIRD YEAR MEDICAL STUDENTS RECOGNISE INTERPRETING FULL BLOOD COUNT RESULTS?

S. Lovato1,2, J. Arnold1,2

Aims: To prospectively examine the association between health-related quality of life and healthcare utilization among patients with AL amyloidosis.

Background: AL amyloidosis is a rare, complex disease associated with significant organ dysfunction, disability, and death. AL amyloidosis patients interact with the healthcare system in a myriad of ways; however, few studies have quantified healthcare utilization (HCU) in this condition.

Methods: A multiple choice test “best of four” containing ten clinical cases including full blood count results was given to the students. According to the Team Based Learning “TBL” model the students completed the test first individually, “i-RAT” and then after discussing the results in small groups “t-RAT”. The topics and the percentage of correct answers are shown in Table 1.

Results: Twenty four students participated. In the i-RAT none of the scenarios were correctly interpreted by 100% of the students, the scenarios interpreted correctly by at least 70% of the students were only two: B12/folate deficiency and iron deficiency; less than 30% of the students could identify CML, NHL and Multiple myeloma; the remaining topics: thalassemic trait, MDS AML, CLL and Multiple myeloma the number of correct answers was the same for i-RAT and t-RAT.

Summary/Conclusions: This group of medical students found it difficult to correctly diagnose some of the haematological conditions presented, even though they had studied all the conditions before, however the use of a “Team Based Learning” approach where students could discuss the cases in small groups did improve their results. Interestingly for two conditions, for CML and Multiple myeloma the number of correct answers was the same for i-RAT and t-RAT, possibly the students who responded correctly during the i-RAT were each 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 author’s knowledge this is the first study on the effect of applying the Team Based Learning method to haematology teaching. This study showed that TBL could be a useful teaching tool to improve teaching of haematological conditions in medical schools, however the size of the sample was small and the results should be validated with a bigger study.

E1468 LONGITUDINAL ASSOCIATIONS BETWEEN HEALTH-RELATED QUALITY OF LIFE AND HEALTHCARE UTILIZATION IN ALamyloidosis

M. Baylis1, T.P. Quock2, S.D. Guthrie2, M.K. White1, K.L. McCausland1, 1Optum, Lincoln, 2Prothena Biosciences Inc, South San Francisco, United States

Background: Light chain (AL) amyloidosis is a rare, complex disease associated with significant organ dysfunction, disability, and death. AL amyloidosis patients interact with the healthcare system in a myriad of ways; however, few studies have quantified healthcare utilization (HCU) in this condition.

Aims: To prospectively examine the association between health-related quality of life and healthcare utilization among patients with AL amyloidosis.

Summary/Conclusions: This group of medical students found it difficult to correctly diagnose some of the haematological conditions presented, even though they had studied all the conditions before, however the use of a “Team Based Learning” approach where students could discuss the cases in small groups did improve their results. Interestingly for two conditions, for CML and Multiple myeloma the number of correct answers was the same for i-RAT and t-RAT, possibly the students who responded correctly during the i-RAT were each 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 author’s knowledge this is the first study on the effect of applying the Team Based Learning method to haematology teaching. This study showed that TBL could be a useful teaching tool to improve teaching of haematological conditions in medical schools, however the size of the sample was small and the results should be validated with a bigger study.

E1467 WHICH HEMATOLOGICAL CONDITIONS CAN THIRD YEAR MEDICAL STUDENTS RECOGNISE INTERPRETING FULL BLOOD COUNT RESULTS?

S. Lovato1,2, J. Arnold1,2

Aims: To prospectively examine the association between health-related quality of life and healthcare utilization among patients with AL amyloidosis.

Background: AL amyloidosis is a rare, complex disease associated with significant organ dysfunction, disability, and death. AL amyloidosis patients interact with the healthcare system in a myriad of ways; however, few studies have quantified healthcare utilization (HCU) in this condition.

Aims: To prospectively examine the association between health-related quality of life and healthcare utilization among patients with AL amyloidosis.

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Methods: A non-interventional, longitudinal online study was conducted among patients with AL amyloidosis who were recruited with assistance from patient advocacy groups. Initial (n=341) and six-month follow-up (n=226) surveys assessed demographics, disease and treatment characteristics, and health-related quality of life (HRQoL), measured by the SF-36v2® Health Survey physical and mental component summary scores (PCS and MCS). HCU (e.g., outpatient visits, hospitalization, treatment to myeloma care room) was measured during the six-month follow-up. Prevalence of HCU and its bivariate associations with patient characteristics were evaluated. Multivariable logistic regression models were used to test for associations between HRQoL and having an ER visit or hospitalization in the past six months.

Results: Overall, visits with specialists and other healthcare providers during the previous six months were nearly ubiquitous (92.0% and 94.6%, respectively). Collectively, 56.0% of patients reported having ≥1 ER visit or hospitalization. ER visits and hospitalizations were not associated with the numbers or types of organs affected by the disease or the duration of disease. There were significant associations between PCS and ER visits (p<0.05) and between both PCS and MCS and hospitalizations (p<0.05 for all) based on multivariable analyses.

Summary/Conclusions: There is a lack of real-world evidence regarding HCU among AL amyloidosis. This research identified longitudinal associations between HRQoL and HCU, indicating there is potential for using HRQoL surveys as screening tools to predict future HCU for AL amyloidosis patients. The development of prediction models for HCU in AL amyloidosis should consider incorporating HRQoL, as well as disease staging and treatment type.

E1469

SAFETY, FEASIBILITY AND EFFECTIVENESS OF ELECTRICAL MUSCLE STIMULATION IN HOSPITALIZED PATIENTS UNDERGOING AUTOLOGOUS OR ALLOGENIC STEM CELL TRANSPLANTATION AND INTENSIVE CHEMOTHERAPY

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Background: Autologous and allogeneic stem cell transplantation (HSCT) or intensive chemotherapy are the only treatment option for many patients with haematological malignancies. Even after complete remission many patients are physically and psychologically impaired because of intensive treatment and weeks of immobilisation. Electrical muscle stimulation (EMS) is a verified training tool to prevent muscle decline in seniors and helps improving physical performance, functioning, and quality of life, indicated by favourable test results in the EMS group. To our knowledge, the study aimed to answer the following questions: What treatment attributes to myeloma patients and the maximum acceptable risk they are willing to accept? What risk-benefit trade-offs characterise patients’ decision-making around the value of new myeloma medicines. For example, to establish what treatments do myeloma patients value? What is the relative importance of different benefits and risks of treatment. The purpose of this study was to assess myeloma patients' preferences for treatment.

Aims: The study aimed to answer the following questions: What treatment attributes do myeloma patients value? What is the relative importance of different 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 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 future healthcare policy decisions and could be incorporated into the Heath Technology Assessment process.

E1470

MYELOMA PATIENT VALUE MAPPING: A DISCRETE CHOICE EXPERIMENT

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Background: Myeloma is a life threatening haematological cancer. Although myeloma is responsive to treatments, there remains no cure. In recent years, there have been improvements in survival due to the use of high dose therapies, stem cell transplant, and other novel therapies. However, while myeloma patients are living longer, they are also living with symptoms and treatment related toxicities. Therefore, myeloma patients face difficult decisions about the benefits and risks of treatment. The purpose of this study was to assess myeloma patient preferences for treatment.

Aims: The study aimed to answer the following questions: What treatment attributes do myeloma patients value? What is the relative importance of different benefits and risks of treatment. The purpose of this study was to assess myeloma patient 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 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 future healthcare policy decisions and could be incorporated into the Heath Technology Assessment process.

E1471

COST-MINIMIZATION ANALYSIS OF RITUXIMAB SUBCUTANEOUS FORMULATION VERSUS INTRAVENOUS ADMINISTRATION OF RITUXIMAB FOR THE TREATMENT OF NON-HODGKIN'S LYMPHOMA IN THE REPUBLIC OF MACEDONIA

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Background: Rituximab, an anti-CD20 monoclonal antibody, in combination with chemotherapy is a standard of care for non-Hodgkin’s lymphoma (NHL), in which subcutaneous (SC) formulation (rituximab SC) is compared with intravenous (IV) infusion, or fixed dose of 1400mg administered as subcutaneous formulation (rituximab SC). Intravenous infusion of rituximab typically last for three to four hours, while subcutaneous application last approximately five to seven minutes. The evidence to support the use of rituximab SC as an alternative to rituximab IV is primarily based on the phase III, randomised, non-inferiority, open-label SABRINA study. Recent studies demonstrated therapeutic and pharmacokinetic non-inferiority of rituximab SC to rituximab IV.

Aims: The aim of the study was to identify and compare the total costs of subcutaneous (SC) vs intravenous (IV) administration of rituximab for the treatment of NHL patients. The total of 220 NHL patients (mean body surface area 1.9 m², middle aged 59.6 years) were enrolled in the study. Evaluated healthcare resources included drug treatment costs, infusion chair occupying cost, active Healthcare Professional time cost and consumable disposals.

Methods: Cost-minimization analysis was used to evaluate pharmacoeconomic impact of the use of subcutaneous vs intravenous administration of rituximab in the treatment of NHL patients. The total of 220 NHL patients (mean body surface area 1.9 m², middle aged 59.6 years) were enrolled in the study. Evaluated healthcare resources included drug treatment costs, infusion chair occupying cost, active Healthcare Professional time cost and consumable disposals.

Results: Direct costs of administering one course of rituximab, including cost of drug, cost of administration and cost of consumables in all treatment phases (premedication, medication and post medication), for intravenous administration of rituximab were 162.1€ compared to 154.6€ for subcutaneous administration of rituximab. Average time for intravenous administration is 6 hours, 12 minutes and 13 seconds, compared to 10 minutes and 13 seconds for subcutaneous administration. Subcutaneous rituximab incurred less non-drug related costs than intravenous rituximab under the observed clinical practice: 14.62€ vs 1.76€ regarding active healthcare professional time and 10.10€ vs 1.2€ as infusion chair occupying cost.

Summary/Conclusions: Subcutaneous administration of rituximab is a cost-saving therapy in comparison with intravenous administration of rituximab for the treatment of NHL patients in the Republic of Macedonia.

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E1472 QUALITY OF LIFE AND ABILITY TO WORK OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH THYROSINE KINASE INHIBITORS

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Background: Thyrosine kinase inhibitors (TKIs) are now standard treatment for chronic myelogenous leukaemia (CML), but little is known about quality of life (QoL) of the patients.

Aims: The purpose of this study is to evaluate QoL of CML patients receiving TKIs, a disease requiring strict daily compliance with taking these drugs orally, as well as regular clinical and biological controls.

Methods: The study included patients with CML followed in three hospitals in west Algeria between 2004 and 2016. The measure of QoL was performed by the tool of functional assessment of chronic illness therapy (Functional Assessment of Chronic Illness Therapy, FACIT) for leukaemia. We have established QoL scores given by the questionnaire, FACIT, consisting of three levels: TOI for leukaemia trial outcome index, FACT-G for general score, and FACT-LEU for the total score of leukaemia. Specific areas of the questionnaire were associated with QoL of patients such as fatigue and ability to work. The correlation between these data 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 malignances and its goal include patients achieve levels of quality of life (QoL) similar that general population. However, studies developed in Europe and United States have shown that patients on long-term follow-up after HSCT reported lower levels of QOL, more unemployment and lower household income than before the procedure. These relationships have not been examined in Mexican HSCT patients.

Aims: To describe the QOL (EORTC-QLQ), level of employment and household income in Mexican patients on follow-up after HSCT

Methods: This was a cross-sectional study with patients ≥18 years old with at least one year of follow up after HSCT at the National Cancer Institute, Mexico. Results: 30 participants were included, with a median age of 34 years (range 25-60), 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% work part-time and 31% had an informal job) and 48% were unemployed (50% could not find a job and 50% did not want to have a job). Finally, 56% had lower household income than before HSCT.

Summary/Conclusions: Mexican patients showed similar or higher levels of QOL in comparison with samples from other countries, with the exception of higher impact in emotional QOL and better social QL in our sample. Additionally, a substantial minority of patients were unemployed and over half had lower household income after HSCT. More work is needed to identify risks associated with changes in QOL, employment status and income among long-term survivors of HSCT.

E1474 ANTHRACYCLINE INCREASES THE RISK OF DEVELOPING DIABETES IN B CELL LYMPHOMA

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Background: Treatments of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) or R-CHOP like regimens have made B cell lymphoma to be one of the most curative hematological malignancies. Among the effective chemotherapeutic agents in B cell lymphoma treatment, anthracycline plays an important role. However, anthracycline associated bone marrow suppression and cardiotoxicity limit its clinical application. Whether anthracycline would further increase the risk of developing diabetes in B cell lymphoma remains unclear.

Aims: The aim of this study was to compare the cumulative incidences of diabetes in B cell lymphoma patients treated with and without anthracycline. We also investigated the dose effect of anthracycline on diabetes development. Additionally, whether anthracycline would increase the severity and complication of diabetes in B cell lymphoma patients were also studied.

Methods: We conducted this population-based study by using Taiwanese National Health Insurance Research Database. From 2004 to 2011, medical records from a total of 3894 B cell patients were analyzed. To understand whether anthracycline therapy was associated with more diabetes in B cell lymphoma, we compared the cumulative incidence of newly diagnosed diabetes between patients with (n=3147) and without (n=937) anthracycline treatments.

Results: Of the 3894 B cell patients with and without anthracycline treatments (p=0.1448). However, anthracycline remained associated with more diabetes [hazard ratio (HR): 1.59; 95% confidence interval (CI): 1.05–2.39; p=0.0278] after adjustment for age, gender, and comorbidities. Moreover, cumulative anthracycline doses of 253-400mg (HR: 1.94; 95% CI: 1.23–3.05; p=0.0438) 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.

Results: Log-rank test did not show the difference of cumulative incidences of newly diagnosed diabetes between B cell lymphoma patients with and without anthracycline treatments (p=0.1448). However, anthracycline remained associated with more diabetes [hazard ratio (HR): 1.59; 95% confidence interval (CI): 1.05–2.39; p=0.0278] after adjustment for age, gender, and comorbidities. Moreover, cumulative anthracycline doses of 253-400mg (HR: 1.94; 95% CI: 1.23–3.05; p=0.0438) and 401-504mg (HR: 1.83; 95% CI: 1.11–3.01; p=0.0180) increased the incidence density of diabetes in a dose-dependent manner (p=0.0438). Notably, patients with and without anthracycline treatment had similarly adapted diabetes complications severity index alteration (0.58±1.89 vs 0.75±1.85; mean±standard deviation), suggesting anthracycline did not deteriorate outcome of diabetes in B cell lymphoma patients (p=0.4924).

Figure 1.
Summary/Conclusions: Anthracycline therapy was responsible for more diabetes and B cell lymphoma in a dose-dependent manner. More intensive blood sugar monitoring and control should be recommended to B cell lymphoma patients, especially those who received anthracycline treatment.

E1475 THE COST-EFFECTIVENESS OF LENALDODIM 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 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 regimens. The risk of progressive disease associated with RD and VD were estimated from available Chinese trials. The efficacy of VCD and the mortality associated with progressive disease after treatments with RD and VD were lacking in China, therefore were estimated from the published international randomized clinical trials. Published quality of life data was adapted to Chinese mMM patients with health utility adjustment. The model took into account (i) drug acquisition costs, (ii) treatment administration costs, (iii) Chinese urban ambulance costs, (iv) adverse events management costs based on a survey of seven MM centers across China, and (v) 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 head-to-head randomized 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 PFS years than VD (¥272,135 vs ¥244,220) than both VD and VCD. The ICERs (1.41) and more discounted lifetime medical costs (¥494,060 vs ¥272,135 vs ¥244,220) than both VD and VCD. The ICERs (1.36). RD was associated with longer discounted lifetime PFS years than 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 head-to-head randomized trials for the efficacy associated with RD, VD, and VCD, to verify the base case analysis.

Summary/Conclusions: The findings of the content validation, pretesting and cognitive interviews indicate that HM-PRO possesses a strong content validity in different HMs, includes all the issues important to these patients and the statements are easy to read, understand and respond to spontaneously. HM-PRO will undergo further psychometric testing to support its psychometric properties across different types of HMs.

E1476 DEVELOPMENT OF A NEW HAEMATOLOGICAL MALIGNANT PATIENT-REPORTED OUTCOME MEASURE FOR USE IN CLINICAL PRACTICE: HM-PRO

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Background: Health-related quality of life (HRQoL) of patients with haematological malignancy (HM) is greatly affected by the disease and the treatment and therefore not fully captured in a systematic manner in the clinical practice.

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 recruited to produce innovative items and rank them by them. 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=76; mean age=61.1 years; SD=15.3; median age=64.9 years; age range=18-98 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-member panel of experts and 7-member panel of patients, rated the items and discussed them for its 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 see the need to add any item.

Summary/Conclusions: The findings of the content validation, pretesting and cognitive interviews indicate that HM-PRO possesses a strong content validity in different HMs, includes all the issues important to these patients and the statements are easy to read, understand and respond to spontaneously. HM-PRO will undergo further psychometric testing to support its psychometric properties across different types of HMs.

E1477 OVARIAN TISSUE CRYOPRESERVATION IN PEDIATRIC AND ADOLESCENT PATIENTS UNDERGOING CANCER CHEMOTHERAPY AND/OR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Ovarian tissue cryopreservation (OTC) and subsequent re-implantation is the only option available for fertility preservation in prepubertal females, but this approach remains unestablished in pediatric and adolescent patients with cancer. After the experience of OTC for more than 200 patients with primary ovarian failure and more than 50 patients with breast cancer in our center over 5 years, we have started OTC for pediatric and adolescent cancer patients since 2015.

Aims: To define safety and benefits of OTC in pediatric and adolescent patients with undergoing cancer chemotherapy and/or hematopoietic stem cell transplantation.

Methods: From December of 2015 to February of 2017, OTC was performed in 6 girls (median age 14 years, range 11-15) 2 patients with myelodysplastic syndrome, 2 with lymphoma, 1 with acute lymphoblastic leukemia, and 1 with carcinoma. To avoid 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 one of a pair of ovarium that was frozen by vitrification method.

Results: Ovarian tissue samples were successfully collected in all 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

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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. Re-implantation of ovarian tissue has not yet been performed.

**Summary/Conclusions:** Although OTC and subsequent re-implantation is experimental, this approach may be the best method for restoration of ovarian function and fertility preservation in pediatric and adolescent cancer patients. A risk of reseeding malignant cells is a problem still to be conquered.

**E1478**

A MULTI-DISCIPLINARY APPROACH TO CHEMOTHERAPY PRESCRIBING AT NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST

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**Background:** Newcastle Upon Tyne Haematology service has made numerous changes in recent years to provide streamlined care for patients, focusing on reduced wait times & improve quality of care. The original pathway was costly in time, involving several waits for the patient: for urgent venepuncture, physician consultation, prescribing of chemotherapy, specialist pharmacist screening of prescriptions & then a separate trip to pharmacy for dispensing. Patients then returned home & waited for a call from the Clinical Nurse Specialist (CNS) to confirm if blood results were appropriate for chemotherapy administration. If a dose adjustment was required the drug was wasted & patients needed to return to hospital for another prescription. Pharmacy waiting times for oral outpatient chemotherapy or supplementary medications are approximately 30 minutes.

**Aims:** We introduced a weekly multi-disciplinary chemotherapy prescribing meeting in 2013 with the aims of improving prescribing safety; minimising time spent prescribing in clinic & reducing patient waiting times. Present at each meeting is a Haematology Specialist Pharmacist, Haematology CNS, Consultant & Specialist Registrar. Chemotherapy is planned a week in advance on ChemoCare (an electronic chemotherapy prescribing package). Chemotherapy is prescribed & immediately screened by the pharmacist; oral chemotherapy is collected from pharmacy by a CNS prior to clinic. All prescription queries are resolved during this meeting. Deferred oral chemotherapy can be returned to pharmacy stock, minimising waste. Intravenous chemotherapy is pre-planned with authorisation on the day of treatment if the patient is fit to proceed.

**Methods:** In order to tailor the care pathway, we focused on delivery of care to myeloma patients receiving oral chemotherapy, including setting up a nurse-led clinic. Data have been collected to assess service impact, particularly on patient satisfaction. The latter was assessed using a patient survey. Between July-Dec 2014, 66 patients received oral chemotherapy in the Myeloma Consultant-led clinic, Lenalidomide based regimens accounted for 86% of the oral regimens prescribed. On average, 7 patients per week were on maintenance therapy. During this period 8% of chemotherapy courses were deferred due to low blood counts or side-effects. Drugs were not wasted due to the pharmacy agreement.

**Results:** A patient satisfaction survey was undertaken from Jan-June 2015, post-introduction of the nurse-led clinic paired with the MDT chemotherapy prescribing meeting. Patients were asked about a wide-range of quality parameters. Results showed 89% of patients noted a reduction in wait times & 89% felt they spent 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 as more efficient.

**Summary/Conclusions:** The MDT approach to prescribing & dispensing oral chemotherapy & supportive medication has streamlined our way of working & led to greater efficiency for both staff & patients. The new model has changed how patients are seen & assessed and minimised drug wastage, an issue we 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 as more efficient.

**E1479**

FINANCIAL TOXICITY OF THE MANAGEMENT OF MULTIPLE MYELOMA

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**Background:** Advances in supportive care and the development of novel treatment methods have helped to double the life expectancy of patients with newly diagnosed multiple myeloma (MM). Financial toxicity is increasingly recognized as adversely affecting the quality of life and medication adherence, and patients with MM might be particularly vulnerable because of extended treatment duration.

**Aims:** Our aim was to measure financial toxicity and its effects on patients undergoing treatment for MM.

**Methods:** Between October 2016, and January 2017, we did a cross-sectional survey of individuals receiving at least 3 months of ongoing treatment for MM at our department. The survey included the 11-item COST measure (financial toxicity score range 0-44). A paper survey was offered to eligible patients on arrival for routine follow-up visits or treatment, and participants were asked to bring along their test results to complete the survey before leaving the department. 104 surveys were collected. The data were postponed by two psychologists. The COST questionnaire was validated with internal consistency (Cronbach’s coefficient) and item correlation (Pearson’s r coefficient) tests, especially those of Quality of life (EORTC QLQ 30).

**Results:** Of 47 patients approached for the study, 44 individuals completed the survey and 40 (91%) were insured. Analysis of the internal consistency of the COST questionnaire showed an overall Cronbach’s alpha coefficient of 0.84. According to COST data, 26 (59, 1%) patients have a score>22. Patients with financial difficulties have a negative impact on their quality of life (P=0, 02, r>0, 32), and low scores of physical and role functioning (P<0,001, r>0, 5), 29 (66%) patients feel financially stressed, and 23 (52, 3%) did not control their financial situation. After a logistic regression, lower household income (P=0,009) and Poor response to treatment (P=0, 0037) were associated with higher financial burden as measured with the COST score.

**Summary/Conclusions:** Despite insurance and free care, financial toxicity is common in many myeloma patients, especially those with lower income and refractory disease. Strengthened collaboration among patients and health-care stakeholders is needed to promote healthcare reforms that promote high value and affordable myeloma care.
Sickle cell disease

E1481

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 [HbSS], or compound heterozygous with β0-thalassemia [HbSβ0]) or “moderate” groups (compound heterozygous for HbS with either HbC [HbSC], or β-thalassemia [HbSβ+]). 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).

Results: Among our patients, 56 (63%) had a “severe” genotype and 32 (37%) 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-value=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.

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 also detectable in patients with a less severe genotype. Longitudinal studies of SCD should include all diseases genotypes, and examine factors that would reduce the risk of cognitive impairment in each subgroup.

Table 1. Predictor variables of interest

<table>
<thead>
<tr>
<th>Predictor variable</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 (4.9%)</td>
<td>0.14</td>
</tr>
<tr>
<td>DSST T-score</td>
<td>47.6 (14.5)</td>
<td>51 (13.4)</td>
<td>0.006</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 (10^9/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) &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Platelet count (10^12/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>321.2 (142.3)</td>
<td>299.2 (149.1)</td>
<td>0.18</td>
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<tr>
<td>Ferritin (ng/mL)</td>
<td>1116.8 (1864.4)</td>
<td>403.4 (1042.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 (0.3)</td>
<td>0.8 (0.2)</td>
<td>0.91</td>
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<tr>
<td>SBP (mm/Hg)</td>
<td>111.3 (13.4)</td>
<td>118.9 (13.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>68.8 (7.7)</td>
<td>73.5 (18.8)</td>
<td>0.02</td>
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<tr>
<td>MAP (mm/Hg)</td>
<td>83.1 (8.4)</td>
<td>86.8 (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>16 (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. ‡ Includes SCI

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 or slowing their 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, cholestatis, liver synthetic capacity, iron overload, viral hepatitis and hemolytic index), TE and liver imaging (ultrasound, MRI-R2*).

Results: 37 adult patients were evaluated: 32% HbSS, 68% HbSB+; median 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 (R²=0.43, p=0.008), ALT (R²=0.42, p=0.01), AST (R²=0.49, p=0.0022), conjugated bilirubin (R²=0.59, p<0.001), ALP (R²=0.51, p=0.002); a positive strong correlation was observed between TE and GGT (R²=0.79, p<0.001), negative moderate correlation with the albumin (R²=0.47, p=0.0048). We found that the group of patients on eritroexechange programmes had a value of stiffness lower than the group transfused (p=0.007). No significant correlation was found between stiffness and LIC (p=0.11, p=0.67). For 24 patients all record were available at time of first observation until last follow up (f.u.): 75% HbSB+, median age 39.5yrs, male 42%, median f.u. 6 yrs, median stiffness 7.3 KPa IQR: 5.3-11.9 KPa. At the first evaluation we documented a significant positive-moderate correlation of TE with serum ferritin (R²=0.43, p=0.037), AST (R²=0.54, p= 0.006), conjugated bilirubin (R²=0.52 values 0.009) and positive-strong correlation with GGT (R²=0.68, p<0.001); these parameters except of ferritin (R²=0.3, p=0.15) and AST (R²=0.39, p=0.058) have maintained the correlation with last f.u.; albumin and ALP showed a significant strong correlation only at f.u. (albumin R²=0.64, p=0.004; ALP R²=0.7, p=0.0017). To remove factors associated with liver fibrosis we also conducted this analysis in the subset of patients HCV negative without liver iron overload: 26 patients; HbSB+ 73%, median age 40.5yrs, male 50%, median f.u. 6 yrs, median values of stiffness 6.1 KPa IQR: 4.6-7.4 KPa. All significant correlations previously described were confirmed also in this group. Three patients in this cohort presented stiffness value according to F4 METAVIR since their first evaluation: all these patients had a severe hepatic damage due to sickle cell disease.

Table 1.
Microstructural analysis of retino-choroid layers using optical coherence tomography in adult patients with sickle cell disease

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Background: Retinopathy is one of the ophthalmological complications of patients with sickle cell disease (SCD), due to microvascular occlusions; occasionally, proliferative sickle cell retinopathy (PSR) can lead to severe vision loss. Aims: a. to analyze macular alterations in patients with Sickle Cell Disease (SCD) by spectral-domain optical coherence tomography (SD-OCT), using the automated software for retinal segmentation; b. to investigate relationship between OCT abnormalities and the severity of proliferative sickle cell retinopathy (PSR); c. to elucidate the role of potentially contributory systemic factors on the development of macular thickening.

Methods: This is a prospective, observational case-control study. Ophthalmological evaluation, fluorescein angiography and SD-OCT were performed. Central and temporal retinal layers were measured by the SD-OCT Automatic Segmentation software. SCD eyes were divided into two groups based on the presence of visible macular thinning areas. Clinical data and blood samples were collected.

Results: Thirty consecutive adult SCD outpatients were studied (median age 38.7±9.89 (M.F 12:18), including 9 patients with Sickle Cell Anemia (SCA), 17 with Sickle Cell β°-Thalassemia and 4 Hbs/Hbc. One Hbs/Hbc patient had no SCD-related events, thus was not considered due to retinal detachment and severe refractive defect. Nineteen out of 59 eyes (32.2%) and 13 out of 30 SCD patients (43%) were noted to have patchy areas of macular thinning on OCT-SD, 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<0.001). SCD eyes with patchy retinal thinning showed shorter closure time (CT) of inner retinal 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). The role of potentially contributory systemic factors on the development of macular thickening was assessed after multivariate regression analysis: 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 capillaryplexus could explain the different retinal layers' damage and the pattern of thinning. No major statistical differences were found between the three sickle genotypes because of variable co-existing genotypes and retinal signs. Ischemia was found to be associated with visual field losses and visual acuity and can lead to irreversible visual loss, regular ocular checkups are essential for SCD patients.

E1483: NON ABLATIVE TISSUE TRANSPLANTATION CONDITIONING WITH TRESOLUFAN IS CURATIVE IN MURINE MODEL OF SICKLE CELL DISEASE

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Background: Hematopoietic stem cell transplantation (HSCT) for patients with sickle cell disease (SCD) is curative, though significant toxicity from myeloablative conditioning is limiting. We have previously developed knock-in mice producing normal (AA) or sickle (SS) human hemoglobin recapitulating severe anemia, hypothenuria and limited lifespan found in SCD. Reduced-intensity conditioning regimens decrease transplant toxicity and are preferable in non-malignant disorders. Novel approaches have been proposed including targeted depletion of stem cells (ACK2), co-stimulation blockade (anti-CD40L), and combination therapy with less toxic alkylating agents (Tresolufan).

Aims: Optimize non-myeoablative 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 Tresolufan (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 chimera was monitored by FACS combined with droplet-digital PCR. Renal tubular function was assessed by measuring urine osmolality, and morbidity animals underwent necropsy to assess organ damage.

Results: Erythroid hyperplasia was noted in the BM of SS, relative to AA mice. Tresolufan, in a dose-dependent manner, decreased BM cellularity and induced cytopenia in AA and SCD mice. AA mice were able to tolerate Tresolufan at non-toxic doses of 3g/kg. In contrast, SS mice were unable to tolerate doses of 3g/kg unless RBC transfused by d+3. At 3g/kg dose, erythroid 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 SCD mice achieving 50% AA in peripheral blood, fertility was preserved, whereas in AA/SS mice only minimal pathologic chimerism was apparent when compared to age-matched controls. ACK2, anti-CD40L, or low-dose radiation, in combination with Tresolufan (3g/kg), failed to improve engraftment. In contrast, increasing Tresolufan to 3.6g/kg resulted in donor-erythroid chimeraism at 3 months post-transplant in all mice, with improvement in hematologic parameters and normalization of hypocellular BM. These animals are currently being observed for fertility, organ toxicity and survival.

Summary/Conclusions: SCD mice closely mimic human disease in phenotype and ablative conditioning intolerance. Tresolufan, at sub-myeoablative dosing, sustained erythroid chimeraism and reversed the SCD phenotype. Our data suggests that pre-transplant conditioning with Tresolufan 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 THROMBOEMBOLIC EVENTS

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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 TE's 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 (SIMEINS LCS) and thromboelastography ROTEM® was performed in order to analyse NATEM CT(Closure Time), MCF (Maximum Clot Firmness), EXTEN CT, MCF and FIBTEM CT. MCF brain imaging was assessed as well as 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 34 patients (57.4%). Twenty-one patients (34%) had overt stroke, 14/21 had recurrent TEE. Only 5/35 patients had a previous history of overt stroke, 1.5 TIA. In the remaining 30/35 patients ischemic lesions were considered SCIs, 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. 14/61 (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...
Summary/Conclusions: The predominance of non-typhoidal Salmonella and other enteric Gram-negatives as the causative agents of invasive bacterial infections in our study is striking. Despite its success in resource-rich settings, penicillin may not be the optimal prophylaxis for sickle cell anemia patients already vaccinated for pneumococcal and Haemophilus influenzae type b in The Gambia. For sickle cell anemia patients with suspected bacterial sepsis, empirical treatment must be effective against both non-typhoidal Salmonella and Staphylococcus aureus, and account for local resistance patterns. As other countries in the region adopt pneumococcal and Haemophilus influenzae type b vaccination programmes, they may see a change in the spectrum of pathogens found in sickle cell anemia patient populations. Local research may be needed to determine appropriate antimicrobial treatment and prophylaxis regimens for patients with sickle cell anemia.

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.Serum 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 IGF-1, 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). When the groups were compared in terms of serum 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 leuko- cyte 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.
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

E1490
EXTENDING ACCESS TO CARE FOR CHILDREN WITH SICKLE CELL DISEASE THROUGH TELEHEALTH
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Background: Sickle Cell Disease (SCD) is the most common inherited blood disorder in the United States and is highly prevalent in South Carolina. Previous work using administrative databases have shown that 25% of affected individuals live in the more rural PeeDee region and seek acute care from community hospitals. As a result, many of these patients have travel >90 minutes for routine SCD care. Due to the difficulty in travel, many patients from this region were seen at a local children's hospital with SCD who were referred from the difficult. Hydroxyurea, the only drug FDA-approved to modify the course of SCD, requires monthly laboratory assessments in the first year and every 3 months in subsequent years. Thus, in addition to concerns with medication side effects, the frequency of visits limits this option for individuals in rural areas with SCD. The primary aim of this pilot study was to evaluate the feasibility and acceptability of using a telehealth clinic to provide SCD care for children living in a designated rural area. The secondary aims were to improve the clinic adherence for patients living in the rural PeeDee region, decrease the burden of care and expense of travel for affected families and improve Hydroxyurea acceptance and uptake.

Methods: The Medical University of South Carolina (MUSC) Center for Telehealth agreed to sponsor the necessary equipment including the video communication system, moveable camera and tele-stethoscope. A regional partner was identified in the target area willing to host the telehealth clinic. Nurses and Advanced Practice Providers were trained in using the equipment and also trained in spleen palpation techniques which cannot be performed using the telehealth system. A local hospital was also identified where routine laboratories can be performed for children seen in the local clinic. Pediatric patients currently seen at the state’s sickle cell network, (SC)2. This approach will be utilized for both routine care as well as acute care through the state sickle cell network, (SC)2.

Results: The pediatric SCD telehealth clinic was initiated in November, 2014 and data reflects the first 16 months of practice. There were originally 21 patients identified from MUSC of whom 4 families declined interest in participation. Additional children were referred and a total of 7 children were enrolled in the pilot program. The local clinic was unable to sponsor hydroxyurea and the patient family had to travel to the state clinic for the medication. At the conclusion of the pilot study in July 2016, 11 of the original cohort of 19 had continued to participate in clinic (defined as attending more than 3 times in the 16-month period). Prior to the telehealth clinic, 10 of the 19 patients had only been seen once in the previous calendar year and 5 of those patients had not been seen in >18 months. Three new patients were started on Hydroxyurea. Two additional referrals to the telehealth clinic were made during the first 13 months (young adults with SCD who had been LTFU for over 3 years). These young adults were seen once by telehealth and then referred to MUSC for the young adult clinic.

Summary/Conclusions: The pediatric SCD telehealth clinic met its primary aim and has continued monthly operations. Hydroxyurea initiation has improved and decreased travel has been welcomed by participating families. Challenges have included equipment issues, difficulties in post-clinic care coordination and assuring caregivers received discharge information. Future directions include a tele-trancsclaral Doppler program from children with SCD at risk for stroke and additional telehealth clinics for adults with SCD that will be utilized for both routine care as well as acute care through the state sickle cell network, (SC)2.

E1491
EMERGING NEED FOR SICKLE CELL DISEASE UNIVERSAL SCREENING IN ITALY, A EUROPEAN COUNTRY WITH INTENSE MIGRATION FLUXES
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Background: Sickle Cell Disease (SCD) is the most common inherited blood disorder in the United States and is highly prevalent in South Carolina. Previous work using administrative databases have shown that 25% of affected individuals live in the more rural PeeDee region and seek acute care from community hospitals. As a result, many of these patients have travel >90 minutes for routine SCD care. Due to the difficulty in travel, many patients from this region were seen at a local children's hospital with SCD who were referred from the difficult. Hydroxyurea, the only drug FDA-approved to modify the course of SCD, requires monthly laboratory assessments in the first year and every 3 months in subsequent years. Thus, in addition to concerns with medication side effects, the frequency of visits limits this option for individuals in rural areas with SCD. The primary aim of this pilot study was to evaluate the feasibility and acceptability of using a telehealth clinic to provide SCD care for children living in a designated rural area. The secondary aims were to improve the clinic adherence for patients living in the rural PeeDee region, decrease the burden of care and expense of travel for affected families and improve Hydroxyurea acceptance and uptake.

Methods: The Medical University of South Carolina (MUSC) Center for Telehealth agreed to sponsor the necessary equipment including the video communication system, moveable camera and tele-stethoscope. A regional partner was identified in the target area willing to host the telehealth clinic. Nurses and Advanced Practice Providers were trained in using the equipment and also trained in spleen palpation techniques which cannot be performed using the telehealth system. A local hospital was also identified where routine laboratories can be performed for children seen in the local clinic. Pediatric patients currently seen at the state’s sickle cell network, (SC)2. This approach will be utilized for both routine care as well as acute care through the state sickle cell network, (SC)2.

Results: The pediatric SCD telehealth clinic was initiated in November, 2014 and data reflects the first 16 months of practice. There were originally 21 patients identified from MUSC of whom 4 families declined interest in participation. Additional children were referred and a total of 7 children were enrolled in the pilot program. The local clinic was unable to sponsor hydroxyurea and the patient family had to travel to the state clinic for the medication. At the conclusion of the pilot study in July 2016, 11 of the original cohort of 19 had continued to participate in clinic (defined as attending more than 3 times in the 16-month period). Prior to the telehealth clinic, 10 of the 19 patients had only been seen once in the previous calendar year and 5 of those patients had not been seen in >18 months. Three new patients were started on Hydroxyurea. Two additional referrals to the telehealth clinic were made during the first 13 months (young adults with SCD who had been LTFU for over 3 years). These young adults were seen once by telehealth and then referred to MUSC for the young adult clinic.

Summary/Conclusions: The pediatric SCD telehealth clinic met its primary aim and has continued monthly operations. Hydroxyurea initiation has improved and decreased travel has been welcomed by participating families. Challenges have included equipment issues, difficulties in post-clinic care coordination and assuring caregivers received discharge information. Future directions include a tele-trancsclaral Doppler program from children with SCD at risk for stroke and additional telehealth clinics for adults with SCD that will be utilized for both routine care as well as acute care through the state sickle cell network, (SC)2.

This approach will both harness the resources of the state to approach SCD and will also use a technology-based approach to increase education of providers.
The plasmatic hemoglobin (cell-free hemoglobin - Hb) was measured by using (Bantu / Bantu + HC) and 54 without (Bantu / Bantu - HC), respectively. The patients were divided into two groups: those with use or without use of hydroxycarbamide (HC); 22 individuals typed of the SNP rs7203560 and the intravascular hemolysis in patients with SCA, suggesting this variant as a genetic marker of clinical evolution. Recent studies showned that the presence of at least one promoter haplotype profile, and in a steady state. The patients were divided into two groups: with HC treatment. Therefore, we performed an analysis to evaluate the association of SNP in the variant of cell-free Hb levels and hemolysis markers commonly used as hemolysis parameters (relative reticulocytes, the enzymes lactate dehydrogenase and aspartate aminotransferase and unconjugated bilirubin), and we found that the individuals genotypic profile was responsible for 50.7% of the variance (P<0.05). This point suggests 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 syndrome is probably related to the role of this genetic variant in the expression of the alpha globin genes. Its promising that ad tolerant analyzes in other ethnic groups and models of hemolytic anemias, such as those of an acquired type, may be 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.

E1493

ASSESSMENT OF INTERNATIONAL DAY HOSPITALS/INFUSION UNITS FOR THE EVALUATION AND TREATMENT OF SICKLE CELL DISEASE
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Background: A Sickle Cell Disease (SCD) Day Hospital is defined as a "dedicated facility for the treatment of SCD uncomplicated painful crises, operating on principle-based pain management". SCD Day Hospital/Infusion Units (SCD-DH/IU) play a positive role in improving pain management, preventing emergency room visits, hospitalizations, and readmissions. No study to date has systematically surveyed the availability, organization, diagnostic tools/therapy provided, and number of SCD patients treated at these facilities as well as compared these facilities' practices based on location.

Aims: To evaluated and compare availability and characteristics of key SCD-DH/IU components with the overarching goal of enhancing and standardizing across facilities, guidelines and standard of care and help supporting the development of alike outpatient-care units at other health care institutions and countries.

Methods: A Web-based survey was developed and link to the survey sent via email in September 2016 and January 2017, to 120 health care providers (80 in the USA and 40 in other countries) identified via the Global Sickle Cell Disease Network as caring for individuals with SCD. Responses were collected between September 4 and February 10, 2017. Data was analyzed by descriptive statistics and T tests using Graphpad.

Results: Fifty seven surveys were completed (41% response rate) by 51 different institutions. Responses from the USA sites, 27 (53%) were, mostly, from long-standing sickle cell institutions in the East, West, and South. Non USA sites, 15 (29%) included Canada, Oman, France, Kuwait, and England. Location of nine sites (18%) was not available. Data from only 42 sites showed: 34 (81%) sites reported having SCD-DH/IU facility. Thirty-one (73%) sites care for 200 or more individuals with SCD, including 17(40%) caring for more than 400 SCD patients. Self-standing units accounted for 30% of SCD-DH/IU, while most (63%) were part of a multi-specialty unit. Only three site operated 24 hours/day, 7 days/week, while 50% of the sites functioned Monday-Friday, 8am-5pm. Half of the SCD-DH/IU sites treat 1-3 SCD patients, 30% treat more than 10 patients. Treatments available at SCD-DH/IU varied among sites. All performed blood tests, but not all were able to provide IV hydration, IV pain management, and blood transfusions. SCD-DH/IU data such as utilization, therapy outcomes, and admissions/admissions were not tracked by 74% of the sites; only 44% have standard post-discharge/follow-up procedures, ¾ of those were Non-USA sites. Most (89%) sites provide individualized care plans for pain management. Only 29% use Patient Controlled Analgesia (PCA). Most 85% allowed direct hospital admission for patients initially evaluated in the SCD-DH/IU. Seven (19%) sites do not have a dedicated provider (MD/PNP) available to triage SCD patients presenting to the SCD-DH/IU. Non-USA sites were far lower in the percent of patients that provide IV hydration and medications, and the rate of patients that provide IV hydration and medications was far lower in the percent of patients that provide IV hydration and medications. Most (89%) sites provide individualized care plans for pain management. Only 29% use Patient Controlled Analgesia (PCA). Most 85% allowed direct hospital admission for patients initially evaluated in the SCD-DH/IU. Seven (19%) sites do not have a dedicated provider (MD/PNP) available to triage SCD patients presenting to the SCD-DH/IU. Non-USA sites were far lower in the percent of patients that provide IV hydration and medications, and the rate of patients that provide IV hydration and medications was far lower in the percent of patients that provide IV hydration and medications.
Table 1.

**Summary/Conclusions:** This is the first study highlighting key healthcare practices 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 HAEMOPEXIN LEVELS IN HAEMOGLOBIN 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 disease, the release of free haem may 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 (Vinci et al., 2016) in association with elevated haem levels, thus suggesting haemopexin decreases due to chronic haemolysis. No data are available in adults with milder sickling disorder haemoglobin SC (HbSC) disease.

#### 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), haem (Bioassay Systems), and haemopexin (Abcam) levels. Statistical analysis was performed with GraphPad Prism v.5 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 13g/dL) and women (Hb below 12g/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±0.48 vs 7.73±0.813 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.

**E1495**

### ASSOCIATION OF TOLL-LIKE RECEPTOR 2 GENE POLYMORPHISM WITH THE INCIDENCE OF BACTERIAL INFECTIONS IN SICKLE CELL DISEASE

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#### Background:
Despite antimicrobial prophylaxis and immunization, bacterial infection remains a leading cause of morbidity and mortality in sickle cell disease (SCD) patients. Functional hyposplenism/asplenia partially explains their susceptibility, since even young SCD children with functional spleen are at raised infectious risk. Toll-like receptors (TLR), that recognize pathogen molecular patterns, are at the forefront of immune protection. The interaction between TLR and infectious diseases in SCD patients has never been explored.

#### Aims:
To evaluate if functional polymorphisms in TLR confer susceptibility/resistance to infections in SCD.

#### Methods:
160 SCD patients followed either in France (n=104) or Senegal (n=56) with recorded history of infections were tested for SNPs in TLR-1, TLR-2, TLR-4, TLR-6 and TLR-10 by TaqMan 5'-nuclease assay for their association with infectious history. Comparisons between groups were evaluated by x² or Fisher exact T-test with Bonferroni corrections of P-value (Pc); associations were measured by odds ratio (OR).

#### Results:
76 patients were positive for at least one bacterial infectious episode (IP) and 84 had no infection (NIP). Eleven IP had more than one episode of infection. Median age was 25 years (range 4-49) for IP and 23 years (range 3-52) for NIP with no distribution bias in gender (P=0.24). All patients had vaccinations against Streptococcus pneumoniae and Haemophilus influenza B, and patients under 10 years had received penicillin prophylaxis. Etiological agent was identified in 58 cases with encapsulated bacteria (EB) occurring in 35; the most common agents consisted of Mycobacterium tuberculosis, Streptococcus pneumoniae, Salmonella spp, Escherichia coli and Klebsiella pneumoniae. Sites of infection included respiratory tract (n=24), bone and joints (n=21), blood stream (n=17), urinary tract (n=11), central nervous system (n=8) and abdominal (n=5).

#### Results:

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs Number</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Minor Alleles</th>
<th>Major Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-2</td>
<td>4696480</td>
<td>TA</td>
<td>AA</td>
<td>0.02</td>
<td>0.98</td>
</tr>
</tbody>
</table>

#### Summary/Conclusions:
rs4696480 TA genotype apparently confers protection against infections especially for EB. Given the previously demonstrated association of AA genotype with exacerbated expression of inflammatory cytokines as well as association of T allele with lower expression of cytokines it is tempting to postulate that TA genotype can be considered as a compromise between deleterious effects of over inflammatory response (TLR-2 AA genotype) and under response (TLR-2 TT genotype) to infectious agents. Such balanced selection effect is probably reflected by the observed deviation from HWE.
Stem cell transplantation - Clinical

E1496
HIGH PROGNOSTIC VALUE OF PRE-SCT MOLECULAR MINIMAL RESIDUAL DISEASE ASSESSMENT BY WT1 GENE EXPRESSION IN AML TRANSPLANTED IN CYTOLOGIC COMPLETE REMISSION
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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 Wilms’ 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 gene expression) and before allo-SCT (81%±25.6% MRD-WT1-negative and 41±22.4% MRD-WT1 positive cases at the pre-SCT workup). We evaluated post-SCT Overall Survival (OS), Disease Free Survival (DFS) and Relapse Rate, according to MRD-WT1 pre-SCT status.

Results: Both post-SCT OS and DFS were significantly better in patients who were MRD-WT1 negative (WT1<250 copies) at the time of SCT compared with those who were MRD-WT1 positive (WT1>250 copies), with a median OS and DFS not reached in the MRD-WT1 negative group and 9 and 8 months, respectively, in the MRD-WT1 positive group (OS log-rank p<0.0001; hazard ratio [HR]: 0.19, 95% confidence interval [95% CI]:0.02-0.73; DFS log-rank p=0.0001; HR=3.73, 95% CI=2.0-6.72). The relapse rate after allo-SCT was 15% (12/81) in pre-SCT MRD-WT1 negative cases and 44% (18/41) in MRD-WT1 positive cases (p=0.0073). 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 for ASCT in high-risk AML.

E1497
GOOD IMMUNOLOGICAL RECONSTITUTION IN ADULTS WITH ACUTE LEUKAEMIA AFTER ALPHA-BETA TCR/CD19+ DEPLETED HAPLOIDENTICAL STEM CELL TRANSPLANTATION (HSCT)
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Background: HSCT using haplo-identical donors has shown good results in pediatric patients but has been associated with an increased risk of acute GVHD. We report the outcome of 31 autologous and 49 allogeneic HSCT in patients transplanted with αβ/CD19-depleted grafts.

Methods: Twenty-four patients (12 CR1; 8 CR2), 12 in advanced-stage disease at transplant. Conditioning consisted of ATG (n=27) or ALL (n=5) entered to study. Twenty were in CR (12 CR1; 8 CR≥2), 13 patients 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]: 0.19, 95% confidence interval [95% CI]:0.02-0.73; DFS log-rank p=0.0001; HR=3.73, 95% CI=2.0-6.72). The relapse rate after allo-SCT was 15% (12/81) in pre-SCT MRD-WT1 negative cases and 44% (18/41) in MRD-WT1 positive cases (p=0.0073). 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.

Results: Both post-SCT OS and DFS were significantly better in patients who were MRD-WT1 negative (WT1<250 copies) at the time of SCT compared with those who were MRD-WT1 positive (WT1>250 copies), with a median OS and DFS not reached in the MRD-WT1 negative group and 9 and 8 months, respectively, in the MRD-WT1 positive group (OS log-rank p<0.0001; hazard ratio [HR]: 0.19, 95% confidence interval [95% CI]:0.02-0.73; DFS log-rank p=0.0001; HR=3.73, 95% CI=2.0-6.72). The relapse rate after allo-SCT was 15% (12/81) in pre-SCT MRD-WT1 negative cases and 44% (18/41) in MRD-WT1 positive cases (p=0.0073). 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 for ASCT in high-risk AML.
tively. For patients who survived more than 100 days, the incidence of chronic graft-versus-host disease (cGVHD) were 14.9±4.0% and 27.4±10.7%, and that of extensive cGVHD were 2.57±0.7%, and 7.8±1.2% at day 1 and year 3 year.  With a median follow up of 20.1 (2.1-70.1) months for alive patients, 3-year estimated overall survival (OS) and failure-free survival (FFS) were both 92±5.7%. Multivariate analysis showed hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score of ≥3 was significantly associated a worse 3-year survival outcome (86.0% vs 50.0%, P=0.035, Hazard ratio [95% Confidence interval]: 6.266 [1.139-34.463]).

Summary/Conclusions: Haplo-identical transplantation without in vitro T-cell depletion conditioning including BU/CY/ATG is a feasible strategy for adult SAA patients, with successful engraftment, acceptable GVHD, and inspiring survival outcomes. HCT-CI might be an outcome predictor in these patients.

E1499

PLERIXAFOR EFFICIENTLY AND SAFELY MOBILIZES PERIPHERAL BLOOD STEM CELLS: HOVON-107 RESULTS IN HLA-IDEN TICAL SIBLING DONORS AND TRANSPLANTED RECIPIENTS

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Background: Plerixafor (PFX) is a reversible inhibitor of stem cell-stroma cell interactions, by interrupting SDF-1 binding to CXCR4. A single subcutaneous (sc) injection of PFX results in direct release of hematopoietic stem and progenitor cells (CD34+/CD45RA-) with limited side effects and therefore could be of advantage for allogeneic stem cell donors.

Aims: We set out to address the feasibility of sc PFX in family donors and their recipients. Feasibility was defined by the percentage of HLA-matched sibling donors harvesting ≥10^6/kg CD34+ cells/kg recipient weight could be harvested after 1 or 2 gifts of PFX (320 μg/kg).

Methods: Currently, data of 23 donors and 23 transplanted patients are available. All donors (16 male; 7 female, median age: 47, range: 24-60) received PFX sc 9-11 hours before stem cell collection. The median age in patients was 50.5±6.4) diagnosed included: AML/MDS RAEB (n=9), ALL (n=3), MM (n=4), Hodgkin/NHL (n=3), CLL (n=2), other (n=2). Transplant conditioning regime was non-myeloablative in 17 patients and myeloablative in 6. Grains obtained after the first gift PFX were analyzed for the total number of CD34+ cells, CD34 subsets: CD34+/CD45RA-/CD90+ and 90- cells, T-cells and distinct CD4+ T cell subsets including regulatory T cells (Treg), Th1, Th2, and Th17 cells. Median cell numbers assessed in 10 G-CSF-mobilized grains were used as controls.

Results: Criteria for feasibility were met in 22 out of 23 donors ≥2×10^9/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 (17%), headache or tingling (17%), fatigue/malagia (17%), CTC grade 3 fatigue was observed in 1 donor, in 2 donors grade 3 clotting occurred during the leukapheresis procedure. All side effects resolved. The median number of CD34+ cells in the graft was 22.4 (11-57) x10^9 after the first gift PFX were analyzed for the total number of CD34+ cells, CD34 subsets: CD34+/CD45RA-/CD90+ and 90- cells, T-cells and distinct CD4+ T cell subsets including regulatory T cells (Treg), Th1, Th2, and Th17 cells. Median cell numbers assessed in 10 G-CSF-mobilized grains were used as controls.

Figure 1.

Summary/Conclusions: It is possible to conduct autotransplants for patients with MS employing non-frozen peripheral blood stem cells and outpatient condition. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

E1501

VEDOLIZUMAB IN STEROID REFRACTORY INTESTINAL GRAFT-VERSUS-HOST DISEASE

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Background: Steroid refractory intestinal graft-versus-host disease (GVHD) is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT), and treatment options are limited. We have previously described successful treatment of this condition with the antibody vedolizumab, targeting the homing of allogeneic T-cells to the intestinal mucosa by inhibiting the binding of T-cell integrin α4β7 to mucosal addressin MadCAM-1.

Aims: Explore outcome of all patients treated with vedolizumab in our department.

Methods: Prospective case series of 13 patients with steroid refractory gastrointestinal GVHD. Patients received 300mg of intravenous vedolizumab at weeks 0, 2 and 6, followed by infusions every 8 weeks if deemed necessary.
Patients were endoscopically evaluated at time of GvHD diagnosis and follow-up. Treatment characteristics are provided in Table 1.

**Results:** All 13 patients experienced clinical responses, which were confirmed by endoscopies and in mucosal biopsies. 10 patients (77%) achieved a clinical response within 28 days, and half of these were complete responses. At last follow-up 10 patients (77%) had achieved sustained complete responses, 2 patients (15%) had responded partially and 1 patient (8%) suffered disease progression. 7 patients (54%) were alive after a median follow up of 35 weeks. The causes of death were transplantation related toxicity, GvHD in other target organs and infectious complications. Increased relative counts of CD25++CD127low regulatory T-cells prior to treatment were observed in peripheral blood of 7 of 9 evaluable patients, and the relative counts decreased in all 7 patients during follow-up.

**Table 1.**

<table>
<thead>
<tr>
<th>Age, median (range)</th>
<th>50 (18-87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from allo-SCT to intestinal GvHD, median (range)</td>
<td>36 days (9-97)</td>
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<tr>
<td>Intestinal GvHD grade prior to graft, median (range)</td>
<td>2.1 (1-4)</td>
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<tr>
<td>Histologic GvHD grade prior to graft, median (range)</td>
<td>2.9 (0-4)</td>
</tr>
<tr>
<td>Doses of prednisone, median (range)</td>
<td>14 days (6-104)</td>
</tr>
<tr>
<td>Observation time, median (range)</td>
<td>35 weeks (12-90)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our results indicate that vedolizumab may effectively treat steroid refractory cases of intestinal GvHD and is well tolerated. The mechanism of action is likely to be inhibition of allo-reactive T-cells interacting with intestinal endothelial cells. It is unclear why regulatory T-cells were initially increased in our steroid refractory GvHD patients and subsequently normalized. This might initiate reflect a response to the alloreactive inflammation and subsequent redistribution to affected tissues and/or its resolution after successful treatment.

E1502

**RISK FACTORS, OUTCOMES AND CHARACTERIZATION OF ‘AUTOLOGOUS GRAFT VERSUS HOST DISEASE’: THE MAYO CLINIC EXPERIENCE**

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1Hematology/Medical Oncology, Mayo Clinic, Rochester, United States

**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 histo-pathological 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 is a retrospective analysis of patients that received ASCT at Mayo Clinic between January 2006 and December 2016. Autologous GvHD was defined as the development of clinical and 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 49.2-72.6) years and the median time from disease diagnosis to ASCT was 3.1 (0.3-9.6) years. The median age at ASCT was 61.9 (range 49.2-72.6) 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. 17 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.

**Table 1.**

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<thead>
<tr>
<th>Age at ASCT</th>
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<td>Conditioning regimen</td>
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Summary/Conclusions: Our findings suggest that autologous GvHD is associated with significant mortality and early initiation of treatment with steroids results in improved outcomes. Further studies into the mechanisms of the disease are warranted.

E1503

**CNS DEMYELINATION AFTER HAPLO-HSCT AND ITS ASSOCIATION WITH THE IGG INTRATECHAL SYNTHESIS INDEX AND ANTI-MYELIN OLIGODENDROCYTE GLYCOPROTEIN ANTIBODY IN CEREBROSPINAL FLUID**

X. Zhao1, Q. Wang1, J. Zhang1, X. Zhu1, Y. He1, L. Xu1, W. Han1, H. Chen1, Y. Chen1, F. Wang1, J. Wang1, Y. Zhang1, X. Mo1, Y. Chen1, Y. Wang1, Y. Chang1, L. Xu1, K. Liu1, X. Huang1, X. Zhang1

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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.

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Summary/Conclusions: Our findings suggest that autologous GvHD is associated with significant mortality and early initiation of treatment with steroids results in improved outcomes. Further studies into the mechanisms of the disease are warranted.
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, CSF and blood myelin basic protein, 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 incidence of the diseases at 100 days, 1 year and 2 years post-transplantation incidence 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 increase 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 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 lymphocytes, monocytes and platelets, as well as the level of immunoglobulins A, G and M +30 days, +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.

E1504

BASELINE CREATININE CLEARANCE AND ALBUMIN ARE POWERFUL RISK FACTORS FOR ALLOGENEIC TRANSPLANTATION RELATED MORTALITY

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1Hematology Division, Chaim Sheba Medical Center, Tel-HaShomer, Ramat-Gan, Israel, 2Department of Hematology, Erasmus University Medical Center, Rotterdam, Netherlands

Background: The course following allogeneic hematopoietic stem cell transplantation (HSCT) varies between individuals. Baseline comorbidities, commonly scored by the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), are important determinants of transplant risk. However, their prognostic utility varies and only partially accounts for transplantation-related mortality (TRM). Standard pre-HSCT laboratory carries objective physiologic information which can be used for TRM risk estimation.

Aims: Determine the value of pre-HSCT estimated creatinine clearance (CrCl), albumin, and alkaline phosphatase (Alk-p) for TRM prediction.

Methods: The study population included 1,217 patients from two European centers. Indications for transplantation and conditioning regimens were diverse. Donors were either HLA-matched sibling donors (54%), matched unrelated donors (30%), or 9/10 HLA-mismatched unrelated donors (15%). The impact of CrCl, albumin, and Alk-p on TRM was evaluated in a univariate and multivariate analysis. Albumin index includes BBB permeability, the IgG index, the CSF IgG intrathecal synthesis index, CSF and blood myelin basic protein, CSF anti-myelin basic protein antibody, CSF and blood anti-myelin oligodendrocyte glycoprotein antibody.

Results: Patients had a median age of 55 years and HCT-CI scores of 0 (24%), 1-2 (39%), and >3 (37%). A cut-off of CrCl<60 ml/min, albumin<3.5 g/dl, and Alk-p>180 IU/l corresponded with 8.8%, 8.3%, and 6.5% of the population, respectively. CrCl and albumin were associated with increased risk and higher cumulative incidence, adjusted for 3.5, HCT-CI, disease status, donor-recipient sex mismatch, donor type, cytomegalovirus serostatus, and conditioning intensity. Relapse was considered a competing event for TRM. The predictive benefit of adding the laboratory markers to the HCT-CI score was estimated by calculating the area under the receiver operating curves (AUC) of TRM prediction models, with and without the laboratory markers.

Conclusion: Pre-HSCT 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.

E1505

CYTOSTEGENIC 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

F. Guolo1,1, P. Mineto1, P. Minetto1, M. Clavio1, F. Galaverna2, D. Guar1, M. Clavio1, D. Guar1, E. Covello1, N. Colombo1, N. Colombo1, F. Ballerini1, F. Ballerini1, M. Miglino1, C. Di Grazia2, A.M. Raio2, R.M. Lemoli2, M. Gobb1, M. Miglino1, R.M. Lemoli2, M. Gobb1, A. Bacigalupo2

1Clinic of Hematology, Department of Internal Medicine (DiMI), University of Genoa, 2Division of Hematology and Bone Marrow Transplantation, IRCCS AOI San Martino-IST, Genova, Italy

Background: Allogeneic bone marrow transplantation (BMT) offers the only chance of cure for patients with advanced acute myeloid leukemia (AML). High levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk in patient transplanted in first complete remission (CR). WT1 expression levels and multicolor flow cytometry (MFC) are the most common tools to evaluate MRD.

Aims: Here, we analyzed the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk in patient transplanted beyond first CR.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rd (CR3). Pre-BMT MRD was evaluated by WT1 expression and MFC. Median age at transplant was 45 years. Disease phase was CR2 in 63 patients (68%) and CR3 in 29 (32%). Risk group according to European Leukemia Net (ELN) at diagnosis was low in 28 patients (30%), intermediate in 44 (48%) and high in 20 (22%). Sixty-six patients (71%) received myeloablative conditioning, whereas 26 (29%) were conditioned with reduced intensity regimen. Stem cell source was HLA-identical sibling donor (27%), haploidentical haplo (HAPLO) in 24 (26%) and alternative donor in 50 (54%). Median follow-up was 64 months (95% CI 39.8-88.2 months). A positive MFC MRD was defined by the presence of at least 1x103 residual leukemic cells at four or eight (since 2011) color flow-cytometry. WT1 copy

Figure 1.

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.
Results: Relapse occurred in 30 patients (33%) and two years non-relapse mortality was 29%. Three-year estimate of OS was 47.9% (median 19 months). The survival probability was significantly affected by donor source (better for HAPLO, p < 0.05), ELN at diagnosis (better for ELN low risk, p < 0.01), MRD status before BMT evaluated with any method (p < 0.01 for WT1-based MRD, 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 risk diagnosis (2-years OS of 62.2% and 52.7% among MFD 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 of relapse (p < 0.01 and < 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).

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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<0.001, P=0.002 respectively). Patients with GVHD had significantly better response than patients without GVHD (88.9% vs 73.8%, 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/PR; 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 (1.2, 2.3, 6.4, 10.1 and ≥5.32). Patients were grouped as low risk (LR) with a score 0 (12%), intermediate risk (IR) score 1-3 (75%) and high risk (HR) ≥3 (13%). The score was significantly associated with early NRM (day 100: 1.1% vs 1.9% vs 9.2 for LR, IR and HR respectively), long term NRM (1-3 years 1.1-1.1% vs 2.9-4.1% vs 15-20%, respectively, p<0.001) (figure 1) and OS (1-5 years 93-78% vs 91-67% vs 73-50% respectively, p<0.001) (figure 2). No significant association was observed with relapse rate.

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)
L. Jeyaraj Nallathambi1, T. Freeman1, S. Williams1, V. Potter1, R. Benjamin1, K. Cuthill1, S. Devereux2, P. Patten1, D. Yallop1, S. kassam1
1Haematology, Kings college hospital, London, United Kingdom

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 Graph Pad Prism version 5.03. A p value <0.05 was considered significant.

Results: 169 patients received HDC-ASCT. The median age was 58 years (23-74). Patient characteristics are shown in the table (See Image). Thirteen patients (7.6%) required ICU admission at a median of 14 days post cell infusion (range 5-85), with all patients having a neutropenic count <1x10⁹/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

Figure 1.
fluid balance. Six patients required multi-organ support (non invasive ventilation/intubation, haemofiltration and inotropic support) and all died. Four patients died within 30 days of HDC-ASCT and had not engrafted neutrophils at the time of death. Two patients died late at day +120 and day +93 post HDC-ASCT.

The latter had both successfully engrafted neutrophils but subsequently became neutropenic. Causes of death were neutropenic sepsis (3), cerebrovascular accidents (1) and acute respiratory distress syndrome (ARDS) (1) versus hosts death (1). By univariate analysis none of the baseline parameters, comorbidities or conditioning regimens were predictive of ICU admission. The only parameter for which there was a trend for significance was baseline cardiac ejection fraction (EF) <50% (p=0.05). Three patients that required ICU has an EF <50% and 2 were on heart failure medications prior to HDC-ASCT. Two of these 3 patients died.

Summary/Conclusions: In this retrospective series, the risk for ICU admission and death following HDC-ASCT was 7.6% and 3.5% respectively. All patients requiring more than one organ support died. The only predictor of ICU admission was baseline cardiac ejection 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).

Figure 1: Results: All patients had chemosensitive disease and before transplantation, 34 patients (83%) were in complete (CR) and 7 (17%) in partial remission (PR). A median number of 4.2×10^6 CD34+ cells/kg (range: 1.60-13.30) were infused. All patients showed engraftment with a median time to achieve an absolute neutrophil count (ANC) >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 had to be transfused. The median duration of hospitalization was 25 days.

The most common grade 3 and 4 toxicities 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%) showed a response of the disease but not in CR.

The first transplantation was performed an median time of 3 months after the start of chemotherapy. Eleven patients (27%) have died (3 DLBCL, 3 HL, 2 MCL, 1 GZL, 1 TCL and 1 FL), all due to lymphoma progression. Thus the 1- and 2-year PFS are 73.2% and 57.9% and the 1- and 2-year OVS 85.4% and 79.4%, respectively.

Summary/Conclusions: In conclusion Benda-EAM is feasible with a quite promising outcome. Currently an international randomized phase II trial comparing Benda-EAM with BEAM is recruiting. So far fifty-five of 110 planned patients are randomized and first results are expected for 2018.

E1511
THROMBOTIC MICROANGIOPATHY WITH CONCOMITANT AGVHD AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: RISK FACTORS, SEVERE OUTCOME AND TREATMENT EXPERIENCE

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT)- associated thrombotic microangiopathy (TA-TMA) is a significant complication after allo-HSCT. acute graft-versus-host disease (aGVHD) is one of the risk factors for the occurrence of TA-TMA, and some patients may develop both. Although there has been sufficient information available on aGVHD and TA-TMA, TMA with concomitant aGVHD after allo-HSCT remains not well understood.

Aims: To explore the possible risk factors for the occurrence and mortality of TMA with concomitant aGVHD and to investigate outcomes and treatments of this disorder after allo-HSCT.

Methods: This study was based on patients who underwent allo-HSCT at Peking University People’s Hospital from January 2008 to December 2016. We included patients who showed refractory diarrhea and underwent 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 risk 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 (1.53%) were diagnosed with TMA with concomitant aGVHD and were enrolled in the case group, and 150 (5.80%) were enrolled in the control group. The two groups matched well with regard to baseline characteristics. Based on the nested case-based control study, grade III-IV aGVHD (P=0.000), AKI (P=0.033) and hypertension (P=0.028) were significant independent risk factors associated with the occurrence of TMA with concomitant aGVHD. Considering the case group only, our data suggested that a haptoglobin level below normal (P=0.013), a maximum volume of diarrhea >2500 ml/d (P=0.015) and bloody diarrhea (P=0.049) were significant markers for death in both univariate and multivariate analysis. Among the case group and control group, the 9-year OS rates were 52% and 81% (P=0.001), respectively; the 9-year DFS rates were 50% and 65% (P=0.345), respectively; the 9-year cumulative incidence rates of NRM were 44% and 16% (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 (PE=0, 62.5%; PE 0, 38.9%; P=0.156) had no significant influence on the patient outcome.

Summary/Conclusions: This study demonstrated that patients diagnosed with TMA with concomitant aGVHD after allo-HSCT had a significantly lower OS, higher NRM, and a lower incidence of relapse. The risk factors associated with the occurrence and mortality of TMA with concomitant aGVHD may help us assess the prognosis of patients. The findings also suggested that PE use may be ineffective to these patients.

E1512
SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE MONITORING BY QUANTITATIVE RT-PCR IN CORE BINDING FACTOR AML ON TRANSPLANTATION OUTCOMES

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Background: Despite the well-defined role of minimal residual disease (MRD) monitoring in core binding factor (CBF)-AML after intensive chemotherapy, 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
CBFB-MYH11 transcript levels at HSCT on transplant outcomes in AML patients with CBF abnormalities. 

Methods: We included 61 AML patients with CBF at diagnosis that underwent their first HSCT in complete remission (CR) from January 2007 through May 2016. Of 61, 19 (31%) had t(8;21) chromosomal translocation and 42 (69%) inv(16)(t;16;16). Disease status at HSCT was CR1 in 19 (31%) and CR2 in 42 (69%). Nine patients received haploidentical, six MUD, 12 MRDneg, 22 MRDpos, and four t(1;19) bone marrow (n=22) and cord blood (n=7). Conditioning regimen was myeloablative in 38 (62%) and reduced intensity in 23 (38%) patients. Donors were matched related (MR) in 24 (38%), matched unrelated (MUD) in 26 (43%), and haploidentical in 4 (7%). Quantitative real-time PCR analysis was performed on reverse-transcribed RNA for the CBFB-MYH11 (Type A) and RUNX1/RUNX1T1 fusion transcripts. Fusion (RUNX1/RUNX1T1 and CBFB-MYH11) 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 | f/A | =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 7 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: Cyogenetic 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 (SA) 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(7;3), t(5;18), t(11;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 months (range 12.3-156.3), the 5-year estimated OS rates were 94.7±5.1%. One patient died of acute GVHD. All patients engrafted and three patients developed delayed graft-failure. The incidence of acute GVHD (grade II) and chronic GVHD occurred in 4 (21%) and 2 (11%) patients, respectively. Among 16 patients with available follow-up data of cytogenetics after transplantation, 14 patients disappeared CAs with donor-type normal chromosome. However, in two patients, same CAs was observed after transplantation; in one patient with three developed delayed CAs (t8, +9, and t1;19), same CAs was sustained at the most recent follow-up of 23.1 months without morphologically dysplastic cells. In another patient with t(5;18), CA did not detected at 39.2 months but reappeared at 67.5 months, and this CA had disappeared again at 79.6 months. None of patients developed MDS or AML after SCT.

Summary/Conclusions: This study showed that long-term transplant outcomes in SA 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 AA.
was 109 days (55-395) vs 271 days (55-449) for PET positive vs PET negative patients, respectively. Mortality post relapse was very low among 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 in 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, chemotherapy toxicity and tumor lysis syndrome (TLS) (Chemotherapy-Related TLS) in patients undergoing allo-HSCT can decrease the tumor burden, and immunotherapy should preferably be started in patients with leukemia with relatively low tumor burden. However, some patients who are MRD-positive may refuse or are unable to receive chemotherapy prior to DLI. Few studies have compared the clinical outcomes of Chemo-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 study 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 (79%) patients 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=0.862), 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 probability of survival (OS) in allogeneic hematopoietic cell transplant recipients (HCT). 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 prognostic value 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=30) 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=2.6) and higher NRM (p=0.015; hazard ratio=3.1) for the non-UCBT group, but not for the UCBT group. There was no significant difference in the cumulative incidences of grade 2 to 4 acute GVHD between the non-UCBT group (41%) and the UCBT group (33%, P=0.51). Similarly, there was no significant difference in the cumulative incidences of grade 2 to 4 acute GVHD between the non-UCBT group (8.2%) and the UCBT group (6.1%, P=0.80). The cumulative incidence of extensive chronic GVHD was significantly higher in the non-UCBT group compared with the UCBT group. (38% vs 3.8%, P=0.001) Although not significant, patients in the non-UCBT group were more likely to have the systemic steroid therapy compared with those in the UCBT group. (54% vs 34%, P=0.084).

E1517

LOW DOSE POSTTRANSPLANTATION CYCLOPHOSPHAMIDE CAN ENHANCE THE PROTECTIVE EFFECT OF ATG/G-CSF ON GVHD: RESULTS OF A PHASE II PROSPECTIVE TRIAL

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Background: Anti-thymocyte globulin (ATG)/granulocyte colony-stimulating factor (G-CSF)-supported 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 higher rates of graft failure. Thus, novel strategies are needed to refine each approach: under BMT 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 limited relapse and favorable survival, albeit with relatively high rates
HEPATITIS B REACTIVATION IN HEMATOPOIETIC STEM CELL TRANSPLANTED PATIENTS: 22 YEARS EXPERIENCE OF A SINGLE CENTRE

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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: Patients and electronic records of 561 patients who received HSCT between 1994 and 2015 at the Bone Marrow Transplantation Centre 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 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 600, respectively. The 1-year probabilities of reactivation in anti-HBc IgG positive patients were 56%; CI, 42%>70%; P<0.001. The 1-year probabilities of reactivation were 41%; CI, 24%>61% for anti-HBc IgG positive male patients and 36%; CI, 14%>64% for female patients.

Summary/Conclusions: The results of our study indicate that HBsAg positive patients undergoing autologous or allogeneic HSCT should receive prophylaxis at least one year post-transplant. 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 alloge neic 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 TRANSPLANTATION

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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 MLD, 37 from MUD, 22 from haplo with PT/CY, and 48 with single-unit CB. The median initial inpatient cost was €49179 (IQR, 37030-66923), the median transfusion cost was €11500 (IQR, 9500-12520), and the median length of initial hospitalization was 55 (IQR, 44-75) days. CB showed significantly higher inpatient cost (median, €69858, P=0.008 vs CB), MLD (median, €36998, P=0.001 vs CB), and MUD (median, €39262, P=0.001 vs CB) (Figure). Also, the transfusion cost was highest in CB (median, €22750) compared with haplo (median, €12866, P=0.001 vs CB), MLD (median, €12689, 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 MLD (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 with or without morbidity, 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.

Figure 1.

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.
plication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Peroxisome proliferator-activated receptor (PPAR)-gamma (γ), a potent anti-inflammatory agent, is a transcription factor belonging to the nuclear hormone receptor super family which may be participating in aGVHD.

**Aims:** To explore the role of PPARγ in aGVHD after allo-HSCT.

**Methods:** 65 patients under allo-HSCT and 10 healthy controls were enrolled in study. Peripheral blood (PB) of patients was collected at 15 days, 30 days, 60 days, and 90 days after allo-HSCT. The mRNA expression of PPARγ, IFNγ, T-bet was detected by the real-time PCR. Furthermore, we conducted mixed lymphocyte reaction (MLR) to detect the proliferation of active lymphocytes under different concentration of PPARγ agonist.

**Results:** Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARγ mRNA in healthy controls were significant lower than that in patients after allo-HSCT within 90 days (P<0.05). The expression of PPARγ mRNA hold steady in non-aGVHD 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 experiment of MLR shows that PPARγ agonist rosiglitazone above concentration of 25μM had dose-dependent inhibition effect to proliferation of lymphocytes.

**Summary/Conclusions:** Low expression of PPARγ is associated with aGVHD occurrence and degree. PPARγ agonist can inhibit the proliferation of lymphocytes, which may be a new way to treat aGVHD.

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**E1521**

HAPLOIDENTICAL TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING REGIMEN COULD SERVE AS AN OPTIONAL SALVAGE THERAPY FOR YOUNGER PATIENTS WITH REFRACTORY OR RELAPSED NON-HODGKIN LYMPHOMA

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**Background:** Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) has a well-established role in the treatment of refractory or relapsed (R/R) aggressive non-Hodgkin lymphoma (NHL). However, whether patients with R/R aggressive NHL, in the absence of appropriate HLA-matched donors, can benefit from haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is yet to be elucidated. Herein, we evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

**Aims:** To evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

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**E1522**

OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA HARBOURING INV(3)(q21;q26.2)/T(3;3)(q21;q26.2) WHO CLASSIFICATION


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**Background:** Acute myeloid leukemia (AML) with inv(3)(q21;q26.2)(t(3;3)q21;q26.2) [inv(3)/t(3;3)] 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 clinical outcome. 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)/t(3;3) remain unclear.

**Aims:** We retrospectively examined the impact of inv(3)/t(3;3) 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)/t(3;3), 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 underwent allo-HSCT. OS was estimated using the Kaplan-Meier method and compared using the log-rank test. Relapse and NRM were considered as competing risk and were compared using the Gray’s test. In a multivariate analysis, the Cox proportional hazard model was used to analyze OS. The following variables: age, sex, disease status at allo-HSCT, and was administrated to 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 experiment of MLR shows that PPARγ agonist rosiglitazone above concentration of 25μM had dose-dependent inhibition effect to proliferation of lymphocytes.

**Summary/Conclusions:** Low expression of PPARγ is associated with aGVHD occurrence and degree. PPARγ agonist can inhibit the proliferation of lymphocytes, which may be a new way to treat aGVHD.
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 Jr Clin Pharmacol. 2010 May; 69(5):484). PG-free MEL (Evomela®) has longer stability in solution, results in a slightly higher systemic exposure compared with standard MEL and eliminates propylene glycol administration during high dose melphalan-based conditioning. This agent was shown to be bioequivalent to conventional melphalan leading to successful 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 12 (50%) and PR in 8 (33%). AHCT was performed entirely as outpatient in 25%.

Summary/Conclusions: These findings revealed that AML with inv(3)/t(3;3) had dismal outcome even after allo-HSCT. Multivariate analysis suggested that myeloablative conditioning regimen might improve the transplant outcome.

Figure 1.

Summary/Conclusions: PG-Free MEL can be safely administered as a single 200mg/m² 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.
remaining, both CD4+ and CD8+ subpopulations remained low and these patients were prone to develop relapse. These findings underscore a putative function of CD8+ T-cells in eliminating post-transplant residual disease and maintaining the patients disease free.

E1525

COMPARISON OF TECAM AND BEAM HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LYMPHOMA: EFFICACY AND TOXICITY

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Background: High-dose chemotherapy conditioning regimens followed by autologous hematopoietic stem cell transplantation (AH SCT) generally provide good results in relapsed and refractory lymphomas. 

Aims: Limited data are available to guide the choice of conditioning regimen before AH SCT for patients with lymphoma. We evaluated the efficacy and safety of TECAM and BEAM conditioning as autologous stem cell support in patients with relapsed/refractory lymphomas.

Methods: From July 2011 to October 2016, 64 pathologically confirmed lymphoma patients underwent AH SCT with BEAM (n=32) or TECAM (n=32) regimens in Hematology Division of Ege University Faculty of Medicine. Patients considered as high risk at diagnosis or with relapsed or refractory diseases were eligible for AH SCT. The two groups were well matched in terms of age, gender, histology. Patients were conditioned with TECAM (tiotepa [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 [60mg/m² x two days]) or BEAM (carmustine [300mg/m² x one day], etoposide [200mg/m² x four days], cytarabine [200mg/m² x four days], 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-HSCT) has increased both overall and progression-free survival of patients with MM, as well as improved quality of life. In most cases, patients in the early post-transplant period have severe toxic and infectious complications of varying severity that requires resource-intensive supportive care. The duration of the period of hematopoiesis hypoplasia is dependent on many factors, and an average of 14-16 days. In turn, the attachment of infectious complications in some cases adversely affect the duration of neutropenia.

Aims: To evaluate the possible association of the immune response genes mutation status to the duration of neutropenia after autologous transplantation of peripheral blood stem cells in patients with multiple myeloma.

Methods: The study included 19 patients with multiple myeloma at the age of 32 to 67 years (median - 52 years) who underwent autologous transplantation of peripheral blood 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 (rs2686841), 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 (Lith, 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-HSCT. 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: 0.60-283.34, p=0.03) and with a predominance of heterozygous mutant allele C of the gene IL1β at position -31 (OR 8.17, 95% CI: 1.03-67.94, p=0.04).

Summary/Conclusions: Our findings point to immune response genes involved in the rate of recovery of hematopoiesis in MM patients after autologous HSCT. Identification of the wild-type allele in intron gene IL17A (G-197TA) and mutant allele in intron gene IL1β (T-31IC) 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 ENGRANAGEMENT IN ALLOGENIC TRANSPLANT PATIENTS WITH FEBRILE NEUTROPIA

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Background: Febrile Neutropenia is very severe and urgent early complication after bone marrow transplantation before engraftment. Infection delays engraftments. In this study we retrospectively evaluated the effect and outcome of Granulocyte transfusion on febrile neutropenia and neutrophil engraftment in patients receiving allogeneic transplantation.

Aims: Between 2015-2016, five patients receiving allogeneic bone marrow transplantation (BMT) were treated with granulocyte transfusion at the time of febrile neutropenia before engraftment. The reasons for the use of the granulocyte transfusion were prolonged febrile neutropenia episode.

Figure 1.

Methods: Five AML patients underwent allogeneic transplantation. Three of them transplanted from match sibling donors, one from unrelated donor, and one from (7/10) mismatch mother (haploidentic transplant). They had febrile neutropenia after transplantation, before engraftment. They were given antibiotics. Before the granulocyte transfusion, on the 13th-18th days of transplantation, their neutrophil counts were 0.03-0.08x10^3/dl.

Results: We started Granulocyte transfusion for three days. Granulocyte was collected from unrelated and same blood groups donors. Mean infused gran-
ACUTE RENAL IMPAIRMENT IN ALLOGENEIC STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE

Aims: We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.

Methods: In this study, 113 patients who underwent HSCT were given defibrotide prophylaxis as 25mg/kg per day in four divided intravenous infusions over 2h, starting on the same day as the pretransplantation conditioning regimen. The mean duration of use of defibrotide is 25 days as a prophylaxis.

Results: In this study, 113 patients were recruited, 66 male patients and 47 female patients, with the average of 9.1 years, range 1-20; 8% infants, 55% children and 37% adolescent. There were 50 patients with thalassemia major, 41 patients with leukemia, 11 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenital dyserythropoietic anemia, one patient with osteopetrosis, four patients with familial hemophagocytic lymphohistiocytosis, two patients with severe immune deficiency and one patient with Kostman syndrome. All transplants were allogeneic. No serious side effects were reported in patients during clinical VOD (Seagate criteria). In these patients, defibrotide dose was increased to a treatment dose of 40-60mg/kg per day. One infant patient with Kostman syndrome died due to hepatic and pulmonary veno-occlusive disease. After 36 months of follow up, 7 patients who developed VOD are being well and no patient have transplant related complications.

Summary/Conclusions: Hepatic veno-occlusive disease, which is caused by hepatoctye and sinusoidal vessel endothelium damage, can occur early after HSCT, and in its severe form, may lead to liver failure, hepatorenal syndrome, portal hypertension, and eventually death from multiorgan failure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk.

E1528
DEFIBROTIDE FOR THE PREVENTION AND TREATMENT OF HEPATIC VENO-OCCULSIVE DISEASE AFTER HEMATOPOETIC STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE

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Background: Hepatic veno-occlusive disease (VOD) is a common and serious complication of hematopoietic stem cell transplantation (HSCT) in children. We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.

Aims: We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.

Methods: In this study, 113 patients who underwent HSCT were given defibrotide prophylaxis as 25mg/kg per day in four divided intravenous infusions over 2h, starting on the same day as the pretransplantation conditioning regimen. The mean duration of use of defibrotide is 25 days as a prophylaxis.

Results: In this study, 113 patients were recruited, 66 male patients and 47 female patients, with the average of 9.1 years, range 1-20; 8% infants, 55% children and 37% adolescent. There were 50 patients with thalassemia major, 41 patients with leukemia, 11 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenital dyserythropoietic anemia, one patient with osteopetrosis, four patients with familial hemophagocytic lymphohistiocytosis, two patients with severe immune deficiency and one patient with Kostman syndrome. All transplants were allogeneic. No serious side effects were reported in patients during clinical VOD (Seagate criteria). In these patients, defibrotide dose was increased to a treatment dose of 40-60mg/kg per day. One infant patient with Kostman syndrome died due to hepatic and pulmonary veno-occlusive disease. After 36 months of follow up, 7 patients who developed VOD are being well and no patient have transplant related complications.

Summary/Conclusions: Hepatic veno-occlusive disease, which is caused by hepatoctye and sinusoidal vessel endothelium damage, can occur early after HSCT, and in its severe form, may lead to liver failure, hepatorenal syndrome, portal hypertension, and eventually death from multiorgan failure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk.

E1529
ACUTE RENAL IMPAIRMENT IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS, A PREDICTOR OF MORTALITY

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Background: Allogeneic stem cell transplant (ASCT) remains the only curative option in many malignant and non-malignant conditions. There remains however a risk of significant morbidity and mortality. One risk, acute kidney injury (AKI), can result from drug toxicity and/or haemodynamic instability from sepsis and/or graft vs host disease (GVHD). Existing reports on the impact of AKI have allowed offering allog-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: To investigate the incidence, causes and consequences of AKI in patients undergoing ASCT, including survival.

Methods: The prospectively maintained database of the South Wales Blood and Marrow Transplant programme which serves 77% of the Welsh population, was interrogated to identify patients undergoing ASCT from January 2010 to December 2015. Patients received ciclosporin as GvHD prophylaxis to 100 days post ASCT and weaned thereafter in the absence of GvHD. Serum creatinine and Marrow Transplant programme which serves 77% of the Welsh population, was used to grade AKI. Causes of AKI were assigned after independent review of clinical notes and relevant laboratory data. Patients undergoing second ASCT were excluded. Statistical analysis was carried out using SPSS, version 23 including COX regression and Kaplan-Meier survival analysis.

Results: A total of 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). Race, sex, and gender, ASCT indication, number of hematopoietic cell rejection events, CMV status, donor sex, stem cell source and conditioning regimen (MA vs RIC) were not statistically significant (p>0.05). Within the first year of ASCT, pre-terminal AKI was noted in 29% (n=23) of all patients dying (n=59) with sepsis accounting for 75% of deaths (n=45) (p<0.05). Of the patients alive, only 11% had chronic renal impairment. Chronic GVHD was associated with these patients (73%) one of whom was dialysis dependent.

Summary/Conclusions: AKI is a very common post ASCT. Chronic renal failure is uncommon in long-term survivors. AKI is however a prominent event preceding death. Consistent with other reports AKI and HLA mismatch correlated with inferior outcomes. Poor survival from AKI probably reflects physiological strain from other complications (e.g. sepsis and GVHD). Early recognition and treatment of AKI are important measures in the supportive care of patients with AKI.
in the low, intermediate, high and very high risk groups, respectively, showing a clear distinction by categories (p=0.038) (figure 1). Refrading release, 44 (28.6%) of 156 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 55%, 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 HEPATIC VENO-OCCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME RECEIVING DEFIBRITODE: POST-HOC ANALYSIS OF EXPANDED-ACCESS PROTOCOL FINAL DATA
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Background: Veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is an unpredictable, potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT) conditioning. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved in the European Union to treat severe hepatic VOD/SOS post-HSCT and in the United States to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT. Prior to approval, defibrotide had been available in the United States via an expanded-access program.

Aims: A post-hoc analysis of final data from the defibrotide expanded-access program was used to explore Day +100 survival post-HSCT based on bilirubin-level categories at the time of study entry.

Methods: Patients in the defibrotide expanded-access program had VOD/SOS diagnosed by investigators using Baltimore criteria (bilirubin ≥2mg/dL and ≥2 of: hepatomegaly, ascites, ≥5% weight gain), modified Seattle criteria (≥2 of: bilirubin >2mg/dL, hepatomegaly, or ascites and/or ≥5% weight gain), or biopsy; bilirubin >2 was not required for modified Seattle criteria or biopsy. MOD (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 (44.6 years); 19% of patients; median age was 16 years in the bilirubin ≥2 to <3 group (53.5% of patients) and 13.5 in the ≥3 to ≤5 group (20.4% of patients); median age in other groups ranged from 15 to 17 years. Kaplan-Meier estimated Day +100 survival in all HSCT patients was 58.9%, with 85.6% in patients with BR <2; other bilirubin groups were older and survival estimates decreased (Table 1). In the pediatric (aged ≤16 years) and adult (aged >16 years) patients, patterns were similar (Table 1). Estimated survival rates were lower for patients with MOD across all groups. Of all 1000 HSCT patients with confirmed VOD/SOS, 210 (21%) had treatment-related AEs (TRAEs). The TRAEs in 22% 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).

Bilirubin (mg/dL) | All HSCT Patients | Age ≤16 Years | Age >16 Years |
---|---|---|---|
<2 | 196 | 85.8% | 133 | 57 | 73.5% |
≥2 to <3 | 535 | 55.5% | 378 | 63.7% | 537 | 66.2% |
≥3 to <5 | 204 | 47.2% | 136 | 39.3% | 84 | 31.4% |
≥5 to <8 | 39 | 53.7% | 22 | 54.2% | 17 | 52.0% |
≥8 | 35 | 39.9% | 11 | 15.3% | 12 | 33.3% |

Summary/Conclusions: This post-hoc analysis found that higher bilirubin levels were generally associated with lower Day +100 survival. These results should be interpreted with caution, as only 1 EBMT criterion was analyzed. MOD was also associated with lower Day +100 survival. The results suggest that diagnosis and treatment of VOD/SOS, before bilirubin becomes markedly elevated, may be associated with improved outcome.

Support: Jazz Pharmaceuticals.

E1533
LONG-TERM FOLLOW-UP OF A PROSPECTIVE TRIAL OF INTENSIFIED CHEMO-IMMUNOTHERAPY WITH AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS AFFECTED BY PERIPHERAL T-CELL LYMPHOMA

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Background: 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: 520 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 were transplanted. 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.

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 treatment to intention, at a median follow-up of 76 months, the estimated 7-years progression-free survival (PFS) and overall survival (OS) were 39% (95% CI, 23% >55%) and 62% (95% CI, 46% >78%) respectively. Auto allografted patients were aged 47 years (15 years range, 2 to 67) and 31/37 were alive at the last follow-up.

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.

E1535 POLYMORPHISM IN TGFB1 GENE PREDISPOSES TO RELAPSE AND DEVELOPMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE GRADES III-IV

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1Laboratory of Molecular Diagnostics, Hungarian National Blood Transfusion Service, 2School of PhD Studies, Semmelweis University, 3Department of Haematology and Stem Cell Transplantation, St. Istvan and St. Laszlo Hospital, 4Department of Pathophysiology, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

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 and is defined as a GvHD transformed growth factor B1 (TGFB1) 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 TGFB1 -1347C>T polymorphism in the outcome of HSCT.

Methods: We examined the association of recipient and donor TGFB1 -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 TGFB1 rs1800496 from genomic DNA LightCypher melting curve analysis (LightCycler 480II, Roche Diagnostics) was performed.

Results: We did not find any association between recipients’ TGFB1 -1347C>T polymorphism and HSCT outcome. However, in patients whose unrelated donors carried homozygous TGFB1 -1347TT variant, aGvHD grades III-IV occurred more frequently (aGvHD grade III-IV: 28.9% vs aGvHD grade 0-2: 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 0-2 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 was 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 were not observed.

Summary/Conclusions: Our findings suggest that TGFB1 -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 STEM CELL TRANSPLANTATION

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Background: Live-vaccines should be avoided in the early period following allogeneic hematopoietic cell transplantation (HCT), due to a possible uncontrollable proliferation of the attenuated strains. The post HCT immune system is severely compromised by pharmacological immunosuppression and disruption of lymphoid tissues by conditioning and donor T cell alloreactivity. Patients frequently lost their antibody-based immunity against measles, mumps, and rubella after receiving allogeneic HCT.

Aims: Here, we studied the dynamics of antibody (AB) titers against measles, mumps, and rubella post-HCT.

Methods: We retrospectively analyzed serological AB titers in 240 patients who underwent allogeneic HCT from related 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 loss of protective AB in the first years for patients given a myeloablative condition (MAC) in comparison to patients with reduced condition (RIC), but the proportion of seropositive patients became more equal over time (Figure 1) displays the percentage of seropositive patients to Measles AB given MAC or RIC during 8 years post-HCT. The proportion of patients who retained protective AB titers at 5y post-HCT was higher in recipients of mobilized peripheral blood compared with bone marrow (BM) grafts (measles p=0.01, mumps=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.

Summary/Conclusions: We found a marked decline of AB titers post-HCT with loss of protection in a substantial proportion of patients. Surprisingly, BM grafts did not provide better AB protection post-HCT, despite their higher content of (donor) plasma cells. Together with the observations that (i) patients with lymphoid malignancies (who have received (B-) lymphocyte targeted therapies prior to HCT) had lower AB levels, while (ii) those given reduced intensity conditioning have a higher percentage of protective AB levels in the first years, our data suggest, that residual host plasma cells significantly contribute to AB production during the first years post-HCT. In opposite, the loss of protective AB levels in later years after transplantation was independent of the toxicity of the conditioning regime and may be a effect of weakening signaling for host plasma cells or late donor alloreactivity.

E1537

MICA AND NKG2D POLYMORPHISMS HAVE A SIGNIFICANT IMPACT ON GRAFT VERSUS HOST DISEASE AFTER HLA-MATCHED HEMATOPOIETIC STEM CELL TRANSPLANTATION.

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Background: MICA (MHC class I polypeptide-related sequence A) is a highly polymorphic gene closely linked to the HLA-B locus. It encodes a cell surface inducible glycoprotein, which mediates an activatory signal towards the NKG2D receptor expressed on NK-cells, CD8+ T-cells and NKT-cells. MICA polymorphisms have been shown to influence NKG2D signaling. Indeed, a methionine to valine change at position 129 in exon 3 categorized the MICA alleles into strong (MICA-129 met) and weak (MICA-129 val) binders of NKG2D receptor. 5 repetitions of OCT with 1 additional nucleotide insertion (G) in exon 5 designed the MICA A5.1 alleles with a premature stop codon. Moreover, NKG2D polymorphisms identified alleles associated with a low (NKC3/C 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, MICAAs.1 and NKC3, NKC4) could influence the incidence of acute and chronic graft versus-host disease (GVHD), 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 GVHD (grade I to IV). Recipient MICA A5.1 heterozygosity is also associated with chronic GVHD (p=0.04) while Recipient MICA-129 val/val donor are at a risk factor of chronic GVHD 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 GVHD 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 older patients treated with the TS Bu (TS cohort) or the RIC Flu/Bu regimen, which is used as standard (ST) for older patients at our center ST cohort.

Aims: To assess its impact on survival, we compared the outcomes of older patients treated with the TS Bu (TS cohort) and the reduced intensity conditioning with Flu/Bu regimen, which is used as standard (RIC cohort) for older patients at our center.

Methods: Patients in the TS cohort received IV Bu 80mg/m2/day on day -13 and -12 and Flu 40mg/m2/d followed by IV Bu daly for 4 days -6 to -3, dose adjusted to achieve a total Bu course AUC of 20,000μmol-min based on PK studies. Patients in the ST cohort received Flu 40mg/m2/day followed by IV Bu daily for 4 days (day -6 to -3) dose corrected 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 2016.

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 a reduction in the disease progression without any increase in the TRM. After adjusting for other covariates, the multivariate analysis for PFS confirmed a reduction in the disease progression without any increase in the TRM.

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 older patients with AML/MDS.

Table 1.
HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH DEPLETION OF TCR AB (+) IN CHILDREN: ERCIYES PEDIATRIC BMT CENTER

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Background: Recently, haploidentical hematopoietic stem cell transplantation (HSCT) poses 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 10 years.

Methods: All children who underwent haploidentical HSCT in our center from December 2012 to February 2017 were included in the study. Total 51 haploidentical HSCT in 44 children (17 relapsed/refractory AML, 9 relapsed/refractory ALL, 4 SAA, 4 HLH, 2 Fanconi aplastic anemia, 2 Griscelli syndrome, 1 JMML, and 5 SCID) were performed. Transplantation-related mortality (TRM) was 13.7%. The regimen included ATG, Fludarabine, Thiopeta, Melphalan. Mycophenolate mofetil (MMF) was given as GvHD prophylaxis if the graft contained >5 x10^6/kg TcR αβ (+).

Results: The mean number of apheresis was 2 (1-7) were mobilized by G-CSF only and bone marrow only was used in 4 pts. The median number of apheresis was 2 (1-7). The median of previous lines was 2 (1-9). The stem cell collection was performed after chemotherapy and G-CSF mobilization in most cases, 19 pts (37.2%) had autologous HSCT. The median follow-up was 7.2 years. There were 19 cases of sMDS/AML. The cumulative sMDS/AML incidence was at 5, 10 and 15 years 2.7%, 4.0% and 5.3% (figure A) in all lymphoma pts, 3.3% at 5,10 and 15y in HL pts, and 2.6%, 4.3% and 6.3% in NHL pts (figure B). There was significantly increased sMDS/AML incidence in pts with 3 previous lines (7.7% vs 1.9% at HR 3.9, p 0.005), in pt’s group with chemoresistant disease (8.1% vs 2.3%, HR 3.5, p 0.05), in CD34+ dose<3.0x10^6/kg (14.3% vs 2.5% at 5y, HR 4.9, p 0.05), in BM reinfused group (13.7% vs 2.5% at 5y, HR 4.7, p 0.05), in patients with prolonged platelet engraftment above 20x10^9/l + 15 days vs 11-16 days vs<10 days (5.4% vs 3.0% vs 0.9%, p 0.05). There was no difference between groups of NHL and HL, with and without radiotherapy, according to the apheresis number or neutrophil engraftment. In multivariate analysis in the whole cohort the independent risk factors were number of previous therapy lines, disease status at ASCT and the speed of platelet engraftment (p<0.05). For NHL only number of previous therapy lines (p<0.05), for HL number CD34+cell was infused, use of BM as the progenitor cell source and disease status (p<0.05).

E1540
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: disease type, high dose radiation was used only in 4 pts, the rest of the patients received other chemother-apy regimens (CPB, thiopeta based, ICE and others). All pts except 4 received peripheral blood progenitor cells (PBPC) with median CD34 dose 8.6x10^6/kg (0.4-115.5). BM was used in 22 cases (in 18 together with PBPC). G-CSF was administered from day +7. Involved or extended field radiotherapy either during preparative therapy or in the period after ASCT was used in 37.7% of pts. With median follow-up 7.2 years there were observed 19 cases of sMDS/AML. The cumulative sMDS/AML incidence was at 5, 10 and 15 years 2.7%, 4.0% and 5.3% (figure A) in all lymphoma pts, 3.3% at 5,10 and 15y in HL pts, and 2.6%, 4.3% and 6.3% in NHL pts (figure B). There was significantly increased sMDS/AML incidence in pts with 3 previous lines (7.7% vs 1.9% at HR 3.9, p 0.005), in pt’s group with chemoresistant disease (8.1% vs 2.3%, HR 3.5, p 0.05), in CD34+ dose<3.0x10^6/kg (14.3% vs 2.5% at 5y, HR 4.9, p 0.05), in BM reinfused group (13.7% vs 2.5% at 5y, HR 4.7, p 0.05), in patients with prolonged platelet engraftment above 20x10^9/l + 15 days vs 11-16 days vs<10 days (5.4% vs 3.0% vs 0.9%, p 0.05). There was no difference between groups of NHL and HL, with and without radiotherapy, according to the apheresis number or neutrophil engraftment. In multivariate analysis in the whole cohort the independent risk factors were number of previous therapy lines, disease status at ASCT and the speed of platelet engraftment (p<0.05). For NHL only number of previous therapy lines (p<0.05), for HL number CD34+cell was infused, use of BM as the progenitor cell source and disease status (p<0.05).

E1541
USE OF DEFIBROTIDE TO TREAT TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY

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Background: Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe early transplant complication which results from endothelial injury and it exhibits characteristics of an atypical hemolytic uremic syndrome. Beyond removal or treatment of precipitating factors and, more recently, treatment with eculizumab, TA-TMA remains a therapeutic challenge. Defibrotide, with marked protective effects on the endothelium and the potential to restore thrombocytic/fibrinolytic homeostasis in small vessels, may be considered a therapeutic option for TA-TAM.

Aims: To analyze our center’s experience in the treatment of TA-TMA with defibrotide.

Methods: We reviewed all cases of TA-TMA treated with defibrotide in our allo- 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 chistocytes per high-power field and thrombocytopenia (≤50x10^9/L or ≤50% of normal baseline). Cases without signs of renal or pulmonary failure were not included (n=27; 10/27 patients in 2008–2013; 9/27 patients in 2014).

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). Co-morbid risk factors at the time of TA- TMA onset were: calcineurin inhibitor treatment in all cases (13 cyclosporin, 4 tacrolimus), acute GVHD grade III/IV in 8 cases, 3 CMV reactivations and 2 severe fungal (1 pulmonary aspergillosis, 1 Scedosporium Plicatilis septicemia) or bacterial (1 E Coli sepsis) infections. Median onset of TA-TAM 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
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 Unit of Hematopoietic Cell Therapy of Hospital of Salamanca. The study is approved by ethics committee and the data collection is confidential and anonymous.

Results: The study included 365 patients candidates for ASCT and 217 healthy sibling donors for Allo-SCT who underwent PBSC mobilization. Neupogen® (22%) than with Nivestim® (33%) had mobilized with Nivestim® and 220 were 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® 3.22±3.64, Nivestim® 2.33±3.14, SD=0.45, p=0.02). The mean of the total CD34+ collected cells was 4.75, SD=4.41 in the Neupogen® and 6.35±6.42 in Nivestim® group (p=0.01), with a larger number of apheresis procedures needed in the Neupogen® group (1.24, SD=0.65 vs 1.24, SD=0.45, p=0.02). The mobilization failure rate was slightly higher with Nivestim® (13%) than with Neupogen® (10%), although it was attributed to a more frequent use of lenalidomide. Most patients underwent ASCT: 87% and 92% in the patients in the Neupogen® and biosimilar groups, respectively. There were no statistically significant differences in hematopoietic recovery and transplanted CD34+ cells.

Table 1.

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<th>Table 1. Multivariate analysis of prognostic factors affecting OS and EFS.</th>
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<td>HCT-CI 0 &amp; CR</td>
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<td>HCT-CI 1 &amp; CR</td>
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<td>HCT-CI ≥2 &amp; CR</td>
<td>0.046</td>
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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.
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 7.02±10^6 for Neupogen® vs 6.26±10^6 for Nivestim® (p=0.002), but the minimal target cell dose (2.10^7/kg) was collected in all donors. 8.5% of donors mobilized with Nivestim® failed to achieve the optimal cell dose (4.10^7/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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<td><strong>Result</strong></td>
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<tr>
<td>Neupogen®</td>
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<td><strong>Total Leukocytes n. (%)</strong></td>
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<td><strong>CD34+ cells in blood (%)</strong></td>
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<td><strong>Total lymphocyte cell count</strong></td>
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<td><strong>Erythrocyte cell count</strong></td>
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Summary/Conclusions: Although prospective data are still required, our study supports that the use of the Filgrastim biosimilar Nivestim® has a similar efficacy and safety as mobilization agent compared with the originator Neupogen®.

E1544

PERIPHERAL BLOOD STEM CELL DONATION IN OLDER SIBLING DONORS: IS IT SAFE?

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Background: The introduction of reduced intensity conditioning regimens has led to an increase in allogeneic haematopoietic stem cell transplantation (HSCT) in older patients with a consequent increase in age of family members who are asked to donate HSCs for them. Such donors are expected to have more comorbidities than younger donors and careful assessment of their suitability to donate is required.

Aims: Our aim was to assess the frequency and nature of issues concerning the eligibility of related peripheral blood stem cell donors seen at Churchill Hospital, Oxford between 2012 and 2016. We wished to examine the influence of age and the nature of any extra interventions required to establish donor suitability.

Methods: For clinical data collection donors’ notes were reviewed and analysed retrospectively. A median follow up was used in all cases for sibling donor selection and screening.

Results: During the study period 90 related donors were screened, of whom 1 declined to proceed because of his concerns regarding G-CSF safety, 2 were excluded due to pre-existing medical conditions and 2 were defined medically inadmissible during work-up, and finally 85 donors donated PBSCs to their relatives (36% of allogeneic HSCT performed at our centre). The median donor age was 51 years (range 25-71, n=17 over 60). Nearly half of the donors (44%) took specific haematology investigations e.g. BMA, molecular studies. Additional imaging studies were performed in 13%. In 16% specialist opinion was sought from other specialties with concerns regarding donor fitness or safety. 13 out of 85 cases were handled as planned deviation from our standard eligibility criteria. 59% travelled abroad, of whom 14% visited a malarial area within a year of donation.

Our aim was to assess the frequency and nature of issues concerning the eligibility of related peripheral blood stem cell donors seen at Churchill Hospital, Oxford between 2012 and 2016. We wished to examine the influence of age and the nature of any extra interventions required to establish donor suitability.

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<tr>
<td><strong>Total Leukocytes n. (%)</strong></td>
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<td><strong>CD34+ cells in blood (%)</strong></td>
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<td><strong>Total lymphocyte cell count</strong></td>
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<td><strong>Erythrocyte cell count</strong></td>
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Figure 1.

Results: A median follow up was 5 months (0.3-63). A median time between allo-HSCT 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 the patients died; 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. Three patients died in patients with MC and in patients with relapses was 78.6% and 26.2%, respectively.

Summary/Conclusions: The prognosis of hematological malignancies is poor if relapse is established after allo-HSCT. DLI protocol as preventive therapy must be created for improving long-term results in high risk patients. 3Prevention is better than cure.

Aims: We have evaluated the safety and efficacy of new conditioning regimen with sequential intensive chemotherap (FLAG-IDA) following by conditioning of Flu-Bu(3).

Methods: The study was designed and developed in two separate transplantation centers in Rui Jin Hospital (RJH, Shanghai) and Institut Paoli-Calmettes (IPC, Marseille) respectively. A total of 47 refractory AML patients with median bone marrow blast of 30.0% (1-90%) and median age 42.2±7.8 years (range 16-62) were enrolled. Thirteen patients received transplantation with mobilized peripheral blood stem cells (PBSC) from HLA-matched sibling donor while 18 and 16 with matched unrelated or haplo-identical donors. All patients received FLAG + 3-days idarubicine (12mg/m2 in RJH or 10mg/m2 in IPC) and then received Flu-Bu regimen (5 days) with IV Busulfan (3-days) with 7-day interval. The GVHD prophylaxis regimens were CsA+MMF+ATG (RJH) or post-cyclophosphamide (IPC).

Results: With a median follow-up of 8 months (1-30 months), a total of 14 patients relapsed with a median time of relapse at 4.8 months (2.1-18.1) and most of the patients relapsed within first 3 months 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±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 significant difference between RJH and IPC in terms of transplantation outcome in univariate analysis.

Summary/Conclusions: Our primary data demonstrated a promising outcome with FLAG-IDA chemotherapy as debulking therapy sequential with Flu-Bu3 conditioning regimen in patients with refractory AML and clinical trial with larger patients cohort is warranted.

E1546
MEMORY T CELLS DONOR LYMPHOCYTE INFUSIONS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION AS A SAFE PROCEDURE TO IMPROVE T-CELL RECONSTITUTION
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Background: Hematopoietic stem cell transplantation (HSCT) is a potential curative treatment for patients with hematologic malignant diseases. Haploidentical transplantation with extensive ex vivo T cell depletion of the graft, has demonstrated to prevent graft versus host disease (GVHD), but the major disadvantage has been the development of graft failure, relapse and infections due to delayed immune reconstitution. A selective T cell depletion method that removes T naïve cells expressing CD45RA+ in haploidentical donor lymphocytes, 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 hematologic diseases with mixed chimerism, severe infections and high risk of relapse after hematopoietic stem cell transplantation.

Methods: Patients with hematologic diseases with poor prognosis who lacked an HLA matched donor were included. The recipients received a CD45RA-depleted haploidentical transplantation, on day 0 they received a first graft with a median CD34+ cell dose of 6.4x10^6/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 6 to 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 levels of mixed chimerism and one had graft failure. These patients were treated with infections of 16 aliquots of cryopreserved CD45RO+ haploidentical donor lymphocytes, the CD45RA+ cells depletion was made using the clinMACS system. The median dose of CD45RO+ cells was 1,02x10^7/Kg, starting at a dose of 1,02x10^7/Kg every 21 days until a median dose of 2,04x10^7/Kg was achieved every 21 days. The CD45RA+ cell dose was a median of 0.0045x10^7/Kg (range: 0-1,63x10^7/Kg). All the procedures were well tolerated, neither adverse events nor GVHD were noticed. After the DLI, a progressive increase in T cells count were observed.

Summary/Conclusions: In our experience DLI enriched for CD45RO+ memory T Cell is a promising and safe strategy for patients with severe viral infections and risk of relapse after haploidentical HSCT, these cells have demonstrated to trigger the CD4 and CD8 T-cell reconstitution, which will help reduce risk infection with a low risk of GVHD. However further studies are needed in order to support this therapy.

E1547
FLAG REGIMEN WITH IDARUBICINE AS CYTOREDUCTION THERAPY BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKEMIA
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Background: Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only curative option for patients with refractory acute myeloid leukemia (AML). However, allo-HSCT with standard conditioning regimen could merely achieve a long-term survival of 20% and the key problem is the high relapse rate even after transplantation.

Aims: To identify the contribution of stutter-bands to the total amount of PCR-product and to derive universal formulas for the chimerism calculation excluding stutter percentage.

Methods: Genomic DNAs of donors and patients were isolated from bone marrow samples. Chimerism was assessed by the STR-PCR analysis (polymerase chain reaction with a panel of primers for loci of short tandem repeats) using OraSure 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 homozygous; 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 time-dependent specific constant (for the same PCR conditions) we derived a formula for recipient DNA percentage: Actual recipient’s %=(apparent rec. total DNA ratio - stuffer/total DNA ratio)*100% (special formulas for hetero- and homozygous on fig. 1). To test these formulae the panel of DNA samples with mixed chimerism from 50 to 97% estimated by independent “stutter free” assay. The results of chimerism estimation based on “stutter-complicated” markers (using proposed formulae) conventional “stutter-free” markers appeared to be the same (SD<1%).

Summary/Conclusions: The use of formulae described may circumvent the absence of the “stuffer-free” informative markers for mixed chimerism estimation.

E1549

INTRODUCING PLERIXAFO TO IMPROVE MOBILIZATION IN MULTIPLE MYELOMA PATIENTS WHO BEHAVE AS POOR-MOBILIZERS IS COST-EFFECTIVE CONSIDERING THE WHOLE MOBILIZATION AND TRANSPLANTATION PROCEDURE

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Background: Plerixafor, a CXCR4-antagonist, is efficient to improve CD34+ cell mobilization and collection in candidates for autologous transplantation who behave as poor-mobilizers. The cost of the drug is however of concern. Published medico-economics studies were mostly conducted in the US, and few including detailed and comprehensive micro-costing of the collection and transplantation process; conclusions may thus not apply to European countries where cost structures are different.

Aims: To compare costs and effectiveness of plerixafor-free and plerixafor-replete management strategies for multiple myeloma patients who behaved as poor-mobilizers after adequate administration of a standard rhG-CSF mobilization regimen.

Methods: Sixty patients diagnosed with multiple myeloma were consecutively identified during years 2009-2011, immediately before and after EMA granted marketing authorization for plerixafor. Poor-mobilizers were defined as having circulating CD34+ cell counts below 20/µL. Plerixafor was introduced or not as marketing authorization for plerixafor. Poor-mobilizers were defined as having CD34+ cells in the control group; however, the proportion of patients who met the criterion “collecting at least 2x10^6 CD34+ cells”; a secondary CEA looked at effectiveness analyses (CEA) were conducted; the primary CEA looked at the number of chemotherapy treatments received before mobilization. Two cost-effectiveness arguments should not been used against the administration of plerixafor in multiple myeloma patients in the European context. Future prospective researches looking at patients reported outcome criteria and poor organization in apheresis facilities are needed.

This work was supported by a grant from SANOFI S.A.
(RBC) recovery, neutrophil and platelet engraftment, pure red cell aplasia (PRCA), acute GVHD, relapse and event-free survival (EFS).

Methods: We retrospectively studied allogeneic transplants performed from January 1, 2013 to December 31, 2016. We collected the baseline variables reflected in Table 1 and analyzed the incidence of HE, neutrophil and platelet engraftments, RBC recovery, PRCA (defined as anemia with transfusion-related red cell transfusion and reticulocytopenia <1% in day +60 without other cytopenias), acute GVHD, relapse of the background disease and survival (at 6, 12 and 24 months) in the ABO compatible groups (ABOc) and in the incompatible (ABOi), the latest divided into major, minor and bidirectional disparity.

Results: A total of 133 transplants were included, with a mean follow-up time of 16.4 months. The median age was 52 years and there were 79 males and 54 females. Diagnoses were mainly AML (n=72), ALL (n=19) and NHL (n=11) (see Table 1). 60 received low intensity and 73 myeloablative regimens. They were HLA identical (n=44), unrelated donor (n=50), haploidentic (n=38) and cord (n=1) and, in most cases, hematopoietic progenitors were obtained from mobilized peripheral blood (90.2%), 4.4% (n=59) presented some type of ABOi: major (n=26), minor (n=25) and bidirectional (n=8). The product was processed in order to prevent hemolysis in only 7 cases (red cell depletion in 4 and deplasmatization in 3). There were 23 hemolytic (18 immediate and 5 delayed) -mostly mild- events, which appeared predominantly in patients with ABO-incompatibility (38.98%) - 50% in major disparity, 28% in minor and 37.5% in bidirectional- vs ABOc (2.7%) and this difference was statistically significant (p<0.0001). No differences were observed in the neutrophil graft between the ABOc group and the ABOi group, nor in the platelet engraftment; in contrast, we found a statistically significant effect on the time to erythrocyte recovery (mean: 49.94 days in 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).

Table 1.

<table>
<thead>
<tr>
<th>Gender</th>
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<th>ABOc (n=44)</th>
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<td>AML, ALL, NHL</td>
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Summary/Conclusions: In our study ABO-incompatible 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 as be well be related to an insufficient study power due to low sample size.

References

E1553

LOW BLOOD CONCENTRATION OF TACROLIMUS CAN BE A RISK OF GRAFT FAILURE AFTER CORD BLOOD TRANSPLANTATION

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Background: Cord blood transplantation (CBT) has recently emerged as an attractive alternative donor. However, graft failure still remains potential threats for morbidity and mortality.

Aims: Several biological mechanisms may contribute to graft failure. Immuno-logical rejection of the graft is known as a major cause of graft failure. Graft failure may also be caused by septicaemia, viral infections, drug toxicity and so on. These events have been frequently occurred just before engraftment, and we often experience fluctuation of blood levels of immunosuppressive drugs. Here, we analyzed an association between blood levels of Tacrolimus (Tac) before neutrophil engraftment and neutrophil engraftment.

Methods: Between January 2011 and July 2016, 76 patients received single-unit CBT at our institutions. We analyzed 59 patients for whom Tac was used for GVHD prophylaxis including Tac and Mycophenolate mofetil (MMF) combination (n=26) and Tac with an additional short Methotrexate (sMTX) (n=33). Sixteen patients who underwent second or third CBT and a patient for whom Tac was not used for GVHD prophylaxis were excluded. We also excluded a patient whose Tac concentration we didn’t check more than two times a week. Tac was started at a dose of 0.02mg/kg/day by continuous i.v. infusion. Tac blood concentrations were monitored at least three times a week before engraftment, and dosages were adjusted to maintain serum levels about 10-20 ng/ml.

Results: Of the 59 patients, 48 patients achieved neutrophil recovery at a median of 22 (range 13-35) days. Two patients died before engraftment from severe PIH and active infection. Nine patients (16.8%) experienced graft failure. Patients who could maintain Tac level above 12ng/ml during the second week after CBT (Tac high group) had an incidence of graft failure of 4.8%, which was significantly lower than the 26.3% seen in the other patients (Tac low group) (p<0.01). Patients for whom Tac and MMF were used (MMF group) had an incidence of graft failure of 3.8%, which was significantly lower than the 36.4% seen in the other patients for whom Tac with an additional sMTX group (MTX group) for GVHD prophylaxis (p<0.01). Combined of these factors, the patients of Tac low group and MTX group had had an incidence of graft failure 40.9%, which was significantly higher than the 5.4% seen in the other patients including Tac high group and MMF group even if the patient were included of Tac low group.

Figure 1.

Summary/Conclusions: Low levels of Tac blood concentration were signifi-cantly 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.

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 unclear.

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 signifi-cantly 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 megalachemotherapy with autologous stem cell transplantation and at time of hematopoietic recovery in patients. Expression of TLR mRNA was assessed by real-time PCR using inventoried TaqMan® Assays from LifeTechnologies/ThermoFisher. Beta glucoronidase (GUSB) served as endogenous control. Reaction was performed in 7500 Real Time PCR instrument (LifeTechnologies) using Gene Expression MasterMix (LifeTechnologies/ThermoFisher). Comparative \( C_T \) 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 the Shapiro-Wilk test. In cases of independent quantitative variables with the normal distribution the statistical analysis took advantage of \( t \) test for unrelated variables. In cases of variables manifesting distribution distinct than the normal one, for independent quantitative variables \( U \) test of Mann-Whitney was used. For dependent quantitative variables of the normal distribution, the \( t \) test for linked variables was applied. In cases of quantitative dependent variables with the distribution distinct from normal, the pair sequence test of Wilcoxon was applied. In order to define a relationships between the studied variables, correlation analysis was performed. Results: 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 \( \Delta C_{TLR2} \) 1.4209±1.0461 vs 1.7877±1.4974 and \( \Delta C_{TLR9} \) 117.853±1.0487 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 \( \Delta C_{TLR9} \) 117.853±1.0487 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 post-VOD/SOS day 10 survival 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 250mg/kg/d in 4 divided doses for a recommended ≥21 days after patients provided informed consent. For these exploratory analyses, Day +100 survival rates in HSCT patients were examined post hoc by time from VOD/SOS diagnosis to start of defibrotide for (1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher’s exact test and (2) patients starting defibrotide on a particular day after diagnosis post-HSCT, using Cochran-Armitage trend test for trend across days. Causes of treatment delay were not assessed.

Results: In the final dataset, timing of initiation date was available for 1000 HSCT patients (512 with MOD) who received ≥1 dose of defibrotide. In 31.0% of all HSCT patients, defibrotide was started the day of diagnosis; in 92.9%, by Day 7. In the population-wide analysis of initiation before/after days 1, 2, 3, 4, 7, and 14 post-diagnosis in both the overall group and MOD subgroup (Figure 1), earlier initiation was associated with significantly higher Day +100 survival rates for all days \( (P<0.001) \), except Day 14 (2.6% of patients started defibrotide after Day 14). The trend test for particular initiation days also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall HSCT group and MOD subgroup \( (P<0.001) \). Adverse events (AEs) and serious AEs occurred in 70.8% and 53.4% of patients, respectively. Other than VOD/SOS and MOD, the most common AE was hypotension (11.7%) and most common serious AE was respiratory failure (7.3%).

Summary/Conclusions: In this exploratory analysis of final study data, earlier defibrotide initiation post-VOD/SOS diagnosis significantly improved Day +100 survival, confirmed by the Cochran-Armitage test \( (P<0.001) \). No specific day provides a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

Support: Jazz Pharmaceuticals.
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 underwent allo-HSCT for different indications at our center, with a median follow up of 12.8 months (0-235) at our center. The population consisted of 59 males and 44 females, the median age was 43.7 years. The RDW was collected from the hemogram at the day of the HSCT cell infusion, before it was performed (table 1). The IBM SPSS STATISTICS program was used for all statistical analyses. Differences were considered statistically significant when p<0.05. The median of RDW values in our study was of 16.4 (11.2-38.5). The areas under the receiver operating characteristic (ROC) curves of RDW were ≤18.4 and >18.4 for the selection of the increased RDW cutoff. We evaluated the association of increased RDW (>18.4) with the development of GVHD. A survival analysis of the association of different levels of increased RDW was performed. A subgroup analysis of the Haploidentical HSCT patients (N=13) was also evaluated.

Results: The presence of increased RDW >18.4 was strongly associated with an increased risk of developing acute GVHD (p=0.009) being present in 80% of the patients. In the haploidentical HSCT subgroup an increased RDW >16 was associated with acute GVHD. (p=0.044). There was no association of chronic GVHD with elevated RDW at day 0 (p=0.563). The survival analysis didn’t found an association of high RDW levels with mortality or survival (p=0.301) but a tendency to an increased survival was show between the RDW level subgroups. (figure2). Where a higher RDW seems to have a better survival, but this should be evaluated in a wider sample.

Summary/Conclusions: RDW at day 0 is a feasible predictor factor of Acute GVHD, most likely as a secondary surrogate marker of inflammation secondary to the conditioning regimen. The presence of other factors contributing to the RDW increase (secondary to other comorbidities) cannot be ruled out; but by itself RDW it’s an easy and affordable prognosis marker for aGVHD that should be further evaluated.

E1556

COMPARISON OF THE BEEAM CONDITIONING REGIMEN AND THE BEAM CONDITIONING REGIMEN IN THE AUTOLOGOUS TRANSPLANTATION 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% BEAM, provided that the differences did not have statistical significance. The CR rate was 63.63% in the BeEAM group versus 50% in the BCU 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.

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 histo-incompatibility between recipient and UCB units and fewer hematopoietic precursors in the product. To improve engraftment rates, especially in larger (i.e. adult) patients (pts), two UCB units can be used. Double UCB transplantation (DUCBT) is being utilized at many centres although it has been noted that, while both units may contribute to engraftment, only one unit becomes “dominant” – i.e. persists to provide long-term hematopoiesis. A variety of predictors of which unit will become dominant have been suggested, primarily the unit that is more closely HLA-matched or the unit with the highest total nucleated cell (TNC) count.

Aims: To determine the likelihood of engraftment, incidence of GVHD, influence of TNC count and HLA mismatch on survival and selection of the dominant cord following DUCBT in adults with high-risk hematologic disorders.

Methods: A retrospective review was performed of adult pts undergoing DUCBT at the referral centre for British Columbia. Recipients signed informed consents for all clinical trials in which they participated. HLA typing at A, B, C and DRB1 loci was done on all pts using high-resolution allele-level testing (HRT). HRT was available at these 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 ≥30x10^6/kg recipient weight. Conditioning 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 lymphoblastic leukemia (n=7), chronic lymphoproliferative disease (n=5), MDS (n=4) or other (n=3). All 31 pts recovered ANC>0.5x10^9/L at median of 20 days (range 14-72). Platelet count reached >20x10^9/L in 26/31 pts at median of 38 days (range 24-188). Acute GVHD developed in 26/31 pts (84%) and chronic GVHD in 17 of the 26 pts (65%) that survived to day +100. Seventeen pts (55%) remain alive, in contin-
vus remission at median follow-up of 3 years (range 0.5-7.0). Ten pts (32%) experienced non-relapse mortality from GVHD (5 pts), infection (4 pts) or unknown cause (1 pt). Four pts (13%) have relapsed at 3.5, 10 and 12 months. Outcomes for pts when the best cord unit match was 0-2 antigen-mismatched (Ag-MM) were superior (8/12 alive and well) to those pts when the best unit was 3 Ag-MM (3/9 alive and well; p=0.20). Unexpectedly, 6/9 pts whose best unit was ≥4 Ag-MM were alive and well. Information on the dominant cord was available on 19 pts (Table 1); in 15/19 pts, the dominant cord was the same or a better HLA match compared to 4/19 with a dominant cord that was an inferior HLA match (p<0.001). However, the TNC was of less importance with the lower TNC unit being dominant as frequently as the higher TNC unit for each HLA match category (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>HLA Match</th>
<th>Higher TNC</th>
<th>Same TNC</th>
<th>Lower TNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Better (n=17)</td>
<td>3/4(4/17)</td>
<td>2/3(2/3)</td>
<td>1/3(1/3)</td>
</tr>
<tr>
<td>Same (n=4)</td>
<td>2/4(2/4)</td>
<td>1/4(1/4)</td>
<td>0/4(0/4)</td>
</tr>
<tr>
<td>Worse (n=4)</td>
<td>1/4(1/4)</td>
<td>0/4(0/4)</td>
<td>0/4(0/4)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: DUCBT is effective in adults with life-threatening hematologic disorders. With current UCB inventories, conditioning therapies and supportive care, graft failure is rare - even in adults. HLA 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.

E1558 CLINICAL ANALYSIS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR 46 ACTIVE RELAPSED AND REFRACTORY ACUTE PEDIATRIC LEUKEMIA

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Background: Given the dismal prognosis for relapsed and refractory (R/R) acute leukemia, many physicians discourage offering hematopoietic stem cell transplantation (HSCT) to adults with hematological malignancies. With current UCB inventories, conditioning therapies and supportive care, graft failure is rare - even in adults. 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.

Methods: We retrospectively analyzed the preliminary outcome of 46 active R/R AML patients who underwent allo-SCT from the same or a different donor.

Aims: To review the management and outcomes of these patients; the incidence of immune reconstitution failure and occurrence of acute GVHD in the immediate post-HSCT period but quickly recovered. The mechanism of tolerance induction using PTCy on the +3, +4 day not limited to deletion of alloreactive T-cell clones, but also affects other leukocyte subpopulations (B cells, monocytes, granulocytes). The use of PTCy at +3, +4 a day is immunologically safe method for prevention of GVHD.

E1560 OUTCOMES OF PATIENTS RELAPSING FOLLOWING ALOLOGenic STEM CELL TRANSPLANTATION FOR AML IN FIRST CR: SINGLE CENTER EXPERIENCE

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Background: Allogeneic stem-cell transplantation (SCT) is a curative therapy for patients with AML but disease relapse continues to be the most common reason for treatment failure. There is no standard therapy for relapse after SCT and treatment results are very poor. Treatment options range from supportive care through chemotherapy and donor lymphocyte infusion (DLI) up to a second SCT from the same or a different donor.

Aims: We report a retrospective study of 36 patients AML relapsed patients following allogeneic stem-cell transplantation in first CR.

Methods: Between 2000 and 2016, 130 patients with AML in first CR underwent allo-SCT. We identified 36/130 patients (27%) who had relapsed and proceeded to review the management and outcomes of these patients; the incidence of relapse was 20% and 54% after myeloablative and reduced intensity conditioning, respectively. The median time to disease relapse after allo-SCT was 11 months (range 5-48). 15/36 (41%) of relapsed patients suffered aGVHD grade II-IV or extensive cGVHD. At time of relapse 15/36 (41%) patients were still taking immunosuppressive treatment, which was immediately suspended.
Results: The patients 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%) received conventional chemotherapy and 4 (22%) received reinduction chemotherapy. In the IT group, 6 (66%) pts achieved a second CR after chemotherapy and DLI/second allo-SCT and 3 (34%) died of treatment toxicity. In the whole patients sample, median overall survival (OS) was 7 months (range 2-74), being 4, 5, 13 months in the PSC, CHT and IT group, respectively. Estimated 1-year and 2-years overall survival was 10%, 15%, 40% and 0%, 0%, 12% in the PSC, CHT and IT groups, respectively. In our experience, 3 independent factors for a longer OS after chemotherapy and immunotherapy have been identified: the absence of previous acute or chronic GVHD (HR=2.7p<0.001), a longer interval between the allo-SCT and relapse than 12 months (HR=1.2, p=0.005) and age less than 40 years (HR=1.3, p=0.005).

Summary/Conclusions: This study shows that salvage chemotherapy (with DLI or second allo-SCT) provides the best results and should be offered, whenever possible, to patients with AML who relapse after allo-SCT performed in first CR. Patients undergoing chemotherapy alone had a poorer outcome. Our results emphasize the need to schedule a prospective protocol combining cytoreductive treatments and immunotherapy in patients in AML relapsing after allo-SCT.

E1561
ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH CHEMOREFRATORY HODGKIN LYMPHOMAS: A RETROSPECTIVE MULTICENTER EXPERIENCE OF THE RETE EMATOLOGICA PUGLIESE (REP)
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Background: Second-line salvage high-dose chemotherapy and autologous stem cell transplantation (SCT) have become the standard of care for refractory/relapsed Hodgkin’s lymphomas (HL), leading to durable responses in approximately 50% of relapsed patients and a minority of refractory patients. Patients with refractory HL after autologous SCT generally have poor clinical outcomes with available therapies and by far, allogeneic SCT represents the only strategy with a curative potential.

Aims: We examined allogeneic transplantation outcomes patients with HL chemorefractory following last salvage treatment.

Methods: 39 patients with HL who received allogeneic SCT in chemorefractory disease, from 2000 to 2016 were retrospectively studied. The median age was 34 years (range 16-57 years) and 23 (59%) were male. The majority of patients (80%) had a prior autologous SCT. Most (90%) patients received reduced intensity conditioning, 59% received matched sibling donor and 41% matched-unrelated donor grafts.

Results: 36 patients survived beyond 100 days and were evaluable for chronic GVHD of whom 22 (61%) remained free of cGVHD and 14 (39%) developed cGVHD. The disease status at day 100 post-transplant was reported in 36 out of 39 evaluable patients. 7 (19%) achieved a CR, 11 (31%) had a PR, 15 (42%) a stable disease and 3 (8%) had progressive disease. Following transplantation 30 (77%) patients have relapsed or progressed at a median time of 12.7 months (range 1-39 months) post-transplant. With a median follow-up of 28 months (range 3-95 months) 97 patients remain alive in complete remission, 2 are in stable disease and 26 have died. The Kaplan-Meier estimates PFS at five years was 18%. 6 patients (18%) died of non-relapse mortality (NRM) at a median of 300 days (range 28 days-40 months) following transplantation. The causes of death included infection (n=2), GVHD (n=3), multi-organ failure (n=1).

Summary/Conclusions: Allogeneic SCT remains to be a viable option for patients who are refractory to salvage chemotherapy, especially because better results are obtained when this treatment is applied earlier. Despite the reduction of NRM and GVHD, disease relapse still represents the major issue in the setting of allogeneic SCT failure. The availability of novel agents resulting in objective responses may eventually result in increased eligibility for allogeneic SCT.

E1562
RESULTS OF THE IMPLEMENTATION OF CRYOTHERAPY IN PROTOCOLS OF ORAL MUCOSITIS PROPHYLAXIS IN PATIENTS SUBJECT TO A TRANSPLANT OF HEMATOPOietIC PROGENITORS. EXPERIENCE OF ONCO-MORO-CR
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Background: Oral mucositis (OM) is one of the main complication during stem cell transplantation (SCT). It has an incidence varies between 47-100%. Numerous prevention strategies have been studied. However, the recommendations of the international guidelines have low evidence to back them up. Cryotherapy is used to reduce OM in conditions that use Melphalan. In our center, we have the cryotherapy implemented in our OM prevention protocol since 2012.

Aims: The main aim is to compare the results in terms of incidence and severity of OM (measured according to World Health Organization scale) in patients in whom cryotherapy was applied and in whom it was not applied as well as the necessity of using morphine and parenteral nutrition. The secondary endpoint is to analyze the occurrence and duration of fever and documentation of infection.

Methods: We examined 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 (2012-2016). Both groups were comparable in baseline characteristics.

Results: The medians were applied since 2012 or 2013 because the measure 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 analysis. Hazard ratios and 95% were estimated with the use of logistic regression model.

Summary/Conclusions: In all patients 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 (30% vs 72%, p=0.149). The use of parenteral nutrition was lower in the non-cryotherapy group (52% vs 43%, p=0.48), but and infection was documented on more occasions in cryotherapy group (27% vs 81%, p=0.04). The median number of days the patients were discharged from the cryotherapy group was lower (+14 vs +15 median days, p=0.39) and the mortality at day 100 was higher in the 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). Hazzar ratio was 0.81 (IC 95% 0.06-0.55).

Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NON-CRYOTHERAPY</th>
<th>CRYOTHERAPY</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F) (%)</td>
<td>53/47</td>
<td>56/44</td>
<td>0.45</td>
</tr>
<tr>
<td>Age Median (years)</td>
<td>32</td>
<td>34</td>
<td>0.45</td>
</tr>
<tr>
<td>CR (%)</td>
<td>20</td>
<td>24</td>
<td>0.48</td>
</tr>
<tr>
<td>VPRD (%)</td>
<td>35</td>
<td>42</td>
<td>0.25</td>
</tr>
<tr>
<td>VPR (%)</td>
<td>13</td>
<td>16</td>
<td>0.48</td>
</tr>
<tr>
<td>VSR (%)</td>
<td>22</td>
<td>24</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Summary/Conclusions: In our center, cryotherapy reduces significantly the severity of mucositis. The use of morphine and parenteral nutrition and other complications do not present such a drastic decline, probably because they influence the gastrointestinal mucositis, which is not combated with cryotherapy. With this results, we are encouraged to continue to include cryotherapy in our protocols.

E1563
REDUCED INCIDENCE OF PRIMARY GRAFT FAILURE IN PATIENTS UNDERGOING HAPLOIDENTICAL STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE
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22nd Congress of the European Hematology Association
636 | haematologica | 2017; 102(s2)
Background: Haploidentical stem cell transplantation (HSCT) is an alternative for patients without HLA matched donors. However, primary graft failure (PGF) and graft versus host disease are still limitations derived from alloreactivity due to HLA mismatch. T cell depleting approaches (in-vivo with post-transplant cyclophosphamide (PT-Cy) or ex-vivo with graft engineering) and surveillance for anti HLA antibodies are strategies intended to reduce these complications. PGF has a high mortality, and treatment with a second graft is not well defined in terms of donor, source, graft engineering or conditioning.

Aims: Our objective is to describe the incidence and risk factors of PGF and treatments if needed.

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 titer donor specific anti HLA antibodies and was desensitized with plasma exchange, Rituximab and IVIG before transplantation. All patients engrafted before day 28 and no PGF diagnosis was established in our serie. We found that 4 patients (3 children, 1 adult) required a boost of CD34 selected graft from the same donor for secondary GF and poor graft function.

Summary/Conclusions: PGF incidence described in literature is 5-10%, we did not find any primary graft failure in our serie. Desensitization therapy appeared to be effective in one patient with anti HLA antibodies. All CD34 boosts were performed for secondary graft failure/poor graft function due to treatment toxicities or viral infections. Unfortunately, analysis of causes and risk factors for secondary GF requires a larger number of patients to be determined.

E1564

RESULTS OF HAPLOIDENTIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is a potentially curative treatment for a variety of hematologic malignancies and nonmalignant hematologic disorders. However, only about a third of candidates for allo-HSCT have HLA-matched siblings. For patients who lack HLA-matched siblings, partially HLA-mismatched (haploidentical) related donors are good alternative sources of stem cells for allo-HSCT.

Aims: In this retrospective, single center study we evaluated safety and efficacy of haploidentical allo-HSCT compared to those of HLA-matched allo-HSCT in patients with lymphoma

Methods: A total of 81 lymphoma patients (Hodgkin and Nonhodgkin) with a mean age of 42 years who underwent allo-HSCT (HLA matched n=46, haploidentic n=35) between July 2010 and July 2016 were analyzed. All patients received Cyclophosphamide (Cy) 50mg/kg i.v. on days +3 and +4. All patients initiated CsA day +5, and then adjusted according to the plasma levels. In addition to CsA, all haploidentical allo-HSCT recipients received MMF until day +35.

Results: There were no significant differences in age, sex, diagnosis, disease status up-front HSCT, or transplant characteristics between the groups except a higher median number of stem cells infused in haploidentical group (p=0.004). The median follow-up was 13 months for haploidentical group and 12 months for HLA-matched group. Outcomes of patients are summarized in Table 1.

Summary/Conclusions: Our results suggest that haploidentical allo-HSCT is a safe treatment modality in patients with relapsed lymphoma who lack HLA-matched siblings. The major problem are seems to be viral infections. Future challenges remain in improving post-transplant immune reconstitution and finding the best approach to reduce the incidence and severity of viral infections, while preserving graft-versus-lymphoma effect to prevent the recurrence of the underlying disease.

E1565

COLLECTION OF PERIPHERAL BLOOD HEMATOPOIETIC PROGENITOR CELLS (PBPC) FROM HEALTHY DONORS: 15 YEARS SINGLE CENTER EXPERIENCE

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Background: hematopoietic stem cell transplantation (HCT) is, nowadays, a consolidated therapy within the treatment of multiple hematological pathologies. In the last two decades, the main method of obtaining hematopoietic progenitor cells is blood leukapheresis after mobilization with granulocytic colony growth factors (G-CSF).

Aims: To describe the experience of our center in apheresis of healthy family donors in the last 15 years. Furthermore, analyze the influence of different variables on the procedure and the yields obtained.

Methods: retrospective analysis was performed on 189 hematopoietic progenitor cell collection (HPCC) from January 2002 to December 2016. The study was carried out at Apheresis Unit, Hospital de La Princesa, Madrid, Spain. Progenitor cells mobilization was performed with G-CSF in all cases at a dose of 10mg/kg b.w. Apheresis device was COBE Spectra in all cases and citrate was the anticoagulant used for all the apheresis procedures. All donors were carefully evaluated and informed on the donation procedure and signed an informed consent for apheresis. The venous access used was mostly peripheral venous access in antecubital veins, and in only 7 cases (3.7%) central venous catheter was required. Donor details studied were age, sex, AB0 group, number of apheresis, number of CD34+ per kilogram collected, and processed volume.

Results: among the 189 donors, 85 were females and 104 were males (45% vs 55%). The hematologic pathologies that motivated transplantation were, in order of frequency, Acute Myeloid Leukemia (AML) (40.2%), Myelodysplastic Syndrome (MDS) (13.8%), Acute Lymphoblastic Leukemia (ALL) (10.1%), Hodgkin’s Lymphoma (HL) (8.5%), Non-Hodgkin’s Lymphoma (NHL) (8.3%), Multiple Myeloma (MM) (5.3%), Chronic Myeloid Leukemia (CML) (4.2%), others 11.8%. A total of 85 donors were related to recipient AB0 group, and 104 donor and recipient had the same group. Median weight of donors was 74 Kg and in recipients was 70.5 Kg. Median age of our donors was 50 and median age of recipients was 51 years. Twenty donors were >65 years (10.6%) and 10 were >70 years (5.3%). Median of processed volume was 13 liters, but if we stratify that volume by recipient’s weight, in those whose were rather than 100 kg, median of processed volume was 18 liters. Two apheresis procedures were performed only in ten donors. Of these, 2 were older than 70 years (20% of total donors over 70 years of age) compared to 8 under 70 years of age (4.5% of all patients in that age range). The median of CD34 + /kg collected was 5 x 10^6. Among the age ranges, median yield of CD34+Kg in patients older than 70 years 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 have 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.
Stem cell transplantation - Experimental

E1566

ALLORESPONSES OF HUMAN T-CELLS FROM ADULT PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD ARE DIFFERENTIALLY IMPACTED BY LENALIDOMIDE - IMPLICATIONS FOR AHSCT

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Background: Immunomodulatory drugs (IMiDs), such as lenalidomide provide a tool to enhance both direct anti-tumor and graft-versus-tumor effects after allogeneic haematopoietic stem-cell transplantation (AH SCT). However, early clinical experience with IMiDs after AHSCT using adult peripheral blood (APB) as a stem cell source has been limited by induction of graft-versus-host disease. Characterization of the mechanisms by which IMiDs can modulate alloresponses of T-cells from different cell sources could facilitate more effective use of these drugs in the setting of AHSCT.

Aims: To use in vitro modelling to identify changes in alloresponses of APB and umbilical cord blood (UCB) T-cells after exposure to the widely used IMiD lenalidomide.

Results: We demonstrate that lenalidomide increases net alloproliferation of APB T-cells by selectively enhancing alloproliferation 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 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.

E1567

USING MARKER GENES ANALYSIS INSTEAD OF MLR ASSAY FOR IDENTIFICATION OF FUNCTIONAL CD4+FOXP3+ REGULATORY T CELLS IN GVHD PROPHYLAXIS


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Background: There are two types of CD4+CD25+Foxp3+ regulatory T cells (Tregs), natural Treg cells (nTreg) developing in the thymus, and induced Treg cells (iTreg) arising from CD4+ naïve T cells. The iTreg cells have been considered important for maintenance of immunological tolerance and correlate with the occurrence of GVHD in some studies. Establishing a quick method to identify the functional iTreg cells is worthy of focusing. Five to ten percent Tregs could be found in human CD4+ T cells and should be expanded via in vitro culture. In order to improve the efficiency of Treg cells for the prevention of GVHD, we attempt to establish a relatively quick analytic method to identify the functional iTreg cells, and then to curtail the iTreg cells harvest time for clinical use. Therefore, using qPCR for marker genes analysis instead of MLR (mixed lymphocyte reaction) assay is an important issue.

Methods: Mouse splenocytes were prepared from mouse spleen. Human PBSC were prepared from peripheral blood (PB) of healthy donors by Ficoll-Hypaque density gradient centrifugation. All T cells were isolated by negative selection, then CD4+naïve T cells were harvested. CD4+ naïve T cells were activated with anti-CD3/CD28 beads in the presence of IL-2, TGF-β and retinoic acid (RA) containing RPM1640 medium. The protocol is showed in Fig. 1.

Results: Seven genes for qPCR analysis were used to identify the functional iTreg cells. We used the different proportions of iTreg cells in total naïve T cells for 7 genes expression analysis and MLR assay to investigate the relationship between different proportion of iTreg cells and naïve T cells expression. Seven genes expression analysis were shown in Fig2. It indicated that the different proportion of iTreg cells could show the different expression profile of these genes. Obviously, the Foxp3 gene expression increased in a great level. Based on our previous
experiments, iTreg cells induction could be TGF-b1 dependent. After different amount of TGF-b1 induction, the genes expression profile also showed the coincidence of the data in Fig.2 (Fig.3). Using the same iTreg populations, MLR assay have been investigated for 5 days. The T cell suppression percentage would be dependent on the iTreg cells proportion (Fig.4A and B). It indicated that the gene expression levels can represent the biological function of iTreg cells. It is the better way to identify the iTreg cells. Further, we have used PBMCs for Treg cell induction, the marker genes expression analysis also showed in Fig.5. After comparing with IL-2 cultured T cells, the gene expressions revealed the difference in between iTreg cells and un-induced T cells.

**Summary/Conclusions:** Our study showed that MLR assay should spend 3 to 5 days for identification of the functional iTreg cells, however, the marker genes analysis took only one day for that. Besides, MLR assay is a more complicated method than qPCR analysis. Using simple analysis for human iTreg cells functional identification could save the time for clinical application and might prevent GVHD occurrence effectively.

**E1568**

**OXIDANT-ANTIOXIDANT SYSTEM IN PATIENTS WITH MULTIPLE MYELOMA**

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**Background:** Multiple myeloma (MM) is one of the most widespread malignant B-cell lymphoproliferative disorders and is characterized by a clonal proliferation of atypical plasma cells in bone marrow or, less frequently, in extramedullary locations synthesizing monoclonal immunoglobulins. Currently, autologous hematopoietic cell transplantation (auto-HSCT) is recognized as the standard method of treatment for young patients (<65 years old) with MM. Moreover, the best auto-HSCT results are observed in patients who have received new medication (thalidomide, bortezomib, and lenalidomide) during induction therapy and who have achieved at least a very good partial response, which leads to a significant increase in overall survival. However, studies reflecting the impact of this kind of treatment on the dynamics of oxidant-antioxidant indicators are virtually non-existent. At the same time, the possibility of treating developing diseases by prescribing medication makes the problem highly relevant.

**Aims:** The aim of the study was to investigate the state of OS-AOS in patients with MM during auto-HSCT.

**Methods:** We studied 20 patients (11 men and 9 women, mean age 49 years) who followed auto-HSCT after high-dose melphalan. The control group consisted of 50 age- and sex-matched healthy persons. The plasma levels of malonic dialdehyde and ceruloplasmin as well as activities of superoxide dismutase and catalase were measured by standard biochemical techniques. In erythrocytes, the level of non-protein thiol groups was studied. The state of OS-AOS was investigated in each patient four times: before and after conditioning with melphalan, at the moment of maximal leukocyte decrease and after complete reconstitution from cytopenia.

**Results:** We have found the features of impaired balance in OS-AOS in MM patients before and as course of auto-HSCT. The level of malonic dialdehyde in MM patients was not significantly different from that in the control group. At the same time, ceruloplasmin plasma level as well as catalase activity were significantly increased in patient group (p<0.05), whereas the level of non-protein thiol groups was decreased in MM (p<0.05). The results of our study have shown, that an imbalance of OS-AOS is frequently seen in MM patients and, possibly, could influence the course of auto-HSCT.

**Summary/Conclusions:** The results of the study indicate a high frequency of occurrence of disturbance of the condition of OS-AOS in patients with MM. The imbalance in the functioning of this system is not entirely eliminated in the process of treating the patients with MM using auto-HSCT. The question of the necessity and methods of the possible correction of OS-AOS in patients with MM, particularly during auto-HSCT, requires further study.

**E1569**

**SURFACE RECEPTOR EXPRESSION PROFILE DEFINES ALLOREACTIVE DONOR CD8+ T-CELLS AFTER MURINE ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION**

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**Background:** Acute graft-versus-host disease (aGvHD) is a severe and often life-threatening inflammatory complication of allogeneic hematopoietic cell transplantation (allo-HCT). aGvHD is mediated by alloreactive donor T cells attacking the gastrointestinal tract, liver, and skin of the host. Efficient strategies to improve aGvHD-related morbidity and mortality will rely on more precise methods than preemptive immunosuppression to consistently predict aGvHD and abrogate disease manifestation without exposing patients to an unwarranted risk for infectious complications. Recent insights into the multistep-pathophysiology of aGvHD provide a good basis for the development of new tests to identify individual patients at risk before the onset of aGvHD.

**Aims:** As pathologic T cell responses rely on spatiotemporally defined programs of T cell activation, acquisition of effector functions, and homing to GvHD target tissues it appeared attractive to assess receptor expression profiles of peripheral blood T cells as potential predictive markers.

**Methods:** Therefore, we characterized the surface receptor expression profile of peripheral blood donor lymphocytes early after allo-HCT in two independent murine models across minor histocompatibility antigens (miHAg) with multimicro 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/B (H-2b, Thy1.2+) and syngeneic C57Bl/6 (9Gy) or BALB/c (H-2d, Thy1.1+) recipients. To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated wild type controls.

**Results:** Comparing a panel of T cell surface receptors, we found the homing markers α4β7 integrin, and P- and E-selectin ligand highly up-regulated on allogeneic peripheral blood donor CD8+ T cells at peak time points of cell migration. The combination of these homing markers with the activation markers CD25 and CD69 at later time points and low expression levels of L-selectin allowed to define alloreactive donor T cells.

**Summary/Conclusions:** Based on this data we propose that alloreactive CD8+ T cells can be identified in miHAg allo-HCT recipients upon their homing receptor expression pattern as soon as six to ten days before the onset of aGvHD.
Thalassemias

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 THALASSAEMIA

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Background: The soluble transferrin receptor (sTfR), that fully reflects the narrow erythropoietic activity, was found to have not only a striking diagnostic accuracy in predicting the risk of extramedullary haematopoesis (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 α genes, a β defect plus a β chain variant. sTfR was measured with a commercially available kit. A group of patients was enrolled in the Myocardial Iron Overload in Thalassaemia (MIOT) network and underwent hepatic iron overload assessment by the T2* Magnetic resonance Imaging (MRI) technique.

Results: The group with homozygous or compound heterozygous for β-thalassemia had the higher sTfR levels. sTfR values were negatively related to age at diagnosis (R=-0.462, P<0.0001), and to age at first transfusion (R=-0.703, P<0.0001). At ROC curve a sTfR>5.3mg/L discriminated the patients with a previous history of occasional transfusions. sTfR values were significantly higher in splenectomized patients. sTfR values were negatively related to age at splenectomy (R=-0.328, P=0.044) and in unsplenectomized patients a significant positive correlation was found between sTfR values and spleen diameter (R=0.572, P<0.0001). sTfR values were negatively related to age at starting chelation therapy (R=-0.564, P=0.044). Patients never chelated showed significantly lower sTfR values than patients under chelation therapy (see Figure). sTfR values were significantly correlated with serum ferritin levels (R=0.321, P<0.0001), but no with LIC values.

Figure 1.

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 THALASSAEMIA MAJOR

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Background: The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta Thalassemia Major (TM).

Aims: The aim of this multicenter study was to assess the distribution of serum ferritin levels in a cohort of well treated TM patients and the possible protective role of really low levels versus iron accumulation in the heart and in the liver.

Methods: We considered 1548 TM patients regularly transfused and chelated consecutively enrolled in the MIOT (Myocardial iron Overload in Thalassaemia network). Myocardial and hepatic iron burdens were quantified by the T2* technique. For the heart a multislice approach was adopted in order to calculate segmental and global T2* values. Hepatic T2* values were converted into liver iron concentration (LIC) values.

Results: Mean serum ferritin levels <500ng/ml were found in 342 (22.1%) patients. Three groups were identified on the basis of mean serum ferritin levels. Both transaminases were significantly lower in patients with serum ferritin <500 ng/ml and between 500 and 1000 ng/ml versus patients with serum ferritin ≥1000 ng/ml. Among patients with serum ferritin <500 ng/ml, 9.1% showed hepatic iron (LIC ≥3mg/g dw). Cardiac and hepatic iron levels were significantly lower in patients with serum ferritin <500 ng/ml than in the other two groups and in patients with ferritin between 500 and 1000ng/ml versus patients with serum ferritin ≥1000 ng/ml (see Figure). Compared to patients with serum ferritin levels<500 ng/ml, the other two groups showed a significant higher risk of cardiac iron overload (odds ratio-OR=2.03, P=0.002 for patients with ferritin 500-1000 ng/ml and OR=5.96, P<0.0001 for patients with ferritin ≥1000ng/ml) and of hepatic iron overload (OR=3.44, P=0.001 for patients with ferritin 500-1000ng/ml and OR=25.43, P<0.0001 for patients with ferritin ≥1000ng/ml).

Figure 1.

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.

E1572
ISCHEMIA MODIFIED ALBUMIN AS A MARKER OF OXIDATIVE STRESS IN CHILDREN AND ADOLESCENTS WITH β-THALASSAEMIA: RELATION TO LIPID PEROXIDATION, IRON OVERLOAD AND VASCULAR DYSFUNCTION

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Background: Patients with β-thalassemia major (β-TM) are under significant iron driven oxidative stress. Ischemia modified albumin (IMA) is an altered type of serum albumin that forms under conditions of oxidative stress and an independent predictor of major adverse cardiovascular events.

Aims: To measure the levels of IMA in 45 children and adolescents with β-TM compared with 30 healthy controls and assess its relation to lipid peroxidation, vascular complications and subclinical atherosclerosis.

Methods: β-TM patients without symptoms of heart disease were studied focusing on transfusion history, chelation therapy, serum ferritin, malondialdehyde (MDA) and IMA levels. Echocardiography was performed and carotid intima media thickness (IMT) was assessed.

Results: IMA and MDA levels were significantly higher in β-TM patients compared with controls (p<0.001). IMA was higher among patients with heart disease and pulmonary hypertension (PH) risk than those without. Serum IMA and MDA levels were elevated among patients with serum ferritin ≥2500 µg/L compared with patients below this cutoff. TM patients compliant to chelation had a significantly lower IMA levels than non-compliant ones. Receiver operating characteristic (ROC) curve analysis revealed that a cutoff value of IMA at 75 U/ml could differentiate β-TM patients with PH risk with 90% sensitivity,
91.4% specificity and positive predictive value of 75% and negative predictive value 97%; area under the curve 0.883 (95% confidence interval 0.752-0.959). In addition, the cutoff value of IMA at 17.5 U/mL could differentiate β-TM patients with heart disease with 80.5% sensitivity, 88.9% specificity and positive predictive value of 96.7% and negative predictive value 73.3%; area under the curve 0.887 (95% confidence interval 0.750-0.962). Significant positive correlations were found between IMA levels and disease duration (r=0.311, p=0.045), white blood cell count (r=0.322, p=0.031), serum alanine aminotransferase (r=0.388, p<0.01) and aspartate aminotransferase (r=0.382, p=0.037). IMA and MDA levels were positively correlated (r=0.503, p=0.001) and there was a significant positive correlation between these two markers and mean serum ferritin (IMA; r=0.545, p<0.001 and MDA; r=0.567, p<0.001) among TM patients. IMA levels were positively correlated to TRV (r=0.621, p=0.008), while negatively correlated to ejection fraction (r=-0.412, p=0.014) and fractional shortening. Both IMA and MDA were positively correlated to CIMT (r=0.607, p<0.001 and r=0.645, p<0.001, respectively).

Summary/Conclusions: Our results highlight the role of oxidative stress in the pathophysiology of vascular complications in thalassemia. IMA could be useful for screening of β-TM patients at risk of cardiopulmonary complications and atherosclerosis because its alteration occurs in early subclinical disease.

E1573

SERUM N-TERMINAL PRO-BRAIN Natriuretic PEPTIDE LEVEL AND ECHOCARDIOGRAPHIC TISSUE DOPPLER ABNORMALITIES IN PATIENTS WITH BETA THALASSEMA 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 immunosorbant assay (ELISA).

Results: Tissue doppler imaging revealed a significant difference of ratio of the early (e') to late (a') right ventricular filling velocities (Rv e'/a' ratio) between cardiac iron overloaded patients reflecting early diastolic dysfunction in cardiac iron overloaded patients. Myocardial performance index of left ventricle (LV_TEI index) by TDI showed significant difference in cardiac iron overloaded patients compared to non cardiac iron overloaded patient (mean±SD = 0.37±0.05 versus 0.55±0.03) 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±72.43.pg/ml versus 18.93±9.65.pg/ml respectively with p-value<0.001) and among cardiac iron overloaded patients compared to non cardiac iron overloaded (mean 212.31±57.18.pg/ml versus 64.75±26.69.pg/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_TD 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 chemotherapy may have diastolic and or systolic dysfunctions that could not be detected by conventional echocardiography but could be highlighted by TDI. CMR, TDI and serum BNP level measurement are promising tools for accurate assessment of cardiac functions and iron overload in thalassemia patients.

E1574

PRENATAL DIAGNOSIS OF HEMOGLOBINOPATHIES IN NORTHERN GREECE: 15 YEARS REPORT

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Background: Hemoglobinopathies constitute the most frequent monogenic disorders worldwide and thalassemias are the most frequent genetic disorders in the Mediterranean basin. In Greece, the frequency of Hb S is 9% and the population 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 covering 52.088 individuals as couples or as individual patients. 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 carried out from 12 weeks of gestation (n=298), in few cases by amniotic fluid sampling (n= 21) collected at 16-18 weeks. Few late carriers 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 / HbA2, β-thal in combination with HbE-Saskaton or D-Punjab, HbE/Hb, Hb E-Saskaton /with carrier of HbS, and Hb O/ Hb O, β-thal or o thal in combination with D Punjab, Hb Brugg/β-thal, silent β-thal silent β-thal. 91% of the couples were of Greek origin, and 9% were immigrants from Romania, Nigeria, Paraguay, Laos, and Thailand. We had an average of 15-32 prenatal diagnosis per year.

Results: The results of DNA analyses of the samples were as follows: 76 fetuses (23%) were found to be homozygote or double heterozygote for clinical significant mutations. These couples were informed of the danger of having an affected child but the termination of pregnancy was left to the couples to decide. Nevertheless all, except three couples, preferred to terminate the pregnancies so we had one case of thalassemia major offspring and two cases of silent β-thal/ O Arab offsprings born. Selective abortion of the affected fetus was performed in the cases of the twin pregnancies (n=6). There have been no cases of misdiagnosed pregnancies and only one obstetric complication (rupture of membrane that lead to miscarriage) was reported.

Summary/Conclusions: It is universally accepted that thalassemia prevention programs are successful in countries with a high frequency of Hb mutations, and prenatal diagnosis is mandatory in all at risk couples. The National Thalassemia Prevention Program has effectively decreased the incidence of thalassemia major and sickle cell syndromes in our country and in our region.

E1575

THE IMPACT OF LIVER STEATOSIS ON THE ABILITY OF SERUM FERRITIN LEVELS TO PREDICT LIVER IRON CONCENTRATION AMONG NON TRANSFUSION-DEPENDENT THALASSEMAIA PATIENTS: A CROSS-SECTIONAL EVALUATION

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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 Thalasemia (NTTD).

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 (LCI) measurements were available for 64 patients (54%) who underwent a magnetic resonance Imaging (MRI) scan within the Myocardial Iron Overload in Thalassaemia (MIST) study.

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 LCI values were comparable (Table 1). At ROC curve analysis, a LFT/AST ratio >0.89 predicted the presence of liver steatosis with a sensitivity=0.872 and a specificity =0.901 (P<0.001). Overall, ferritin levels positively correlated with LCI values (R=0.558, P<0.0001) but in patients without steatosis there...
was a strong relationship between ferritin and LIC values (R=0.656, P<0.0001) while in patients with steatosis the correlation was moderate (R=0.426, P=0.05).

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 MFI 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 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 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 characterized by the over-proliferation and lysis/removal of erythroid precursors. This situation is common in thalassemia, mainly in non transfusion-dependent patients (NTDT).

Aims: The present study was designed i) to evaluate the behaviour of cfDNA in IE caused by beta-thalassemia, and ii) to assess whether cfDNA could be useful to quantify IE.

Methods: We studied 49 beta-thalassaemia intermedia (TI) patients (mean age 41 years, range 16-65), 23 of whom were splenectomized. No evidences of tumor, trauma or autoimmune diseases have been observed in any patient at time of the study. Eighteen healthy subjects were also included as control group. The study was approved by the local ethical committee. DNA was extracted by QIAgen silica-based micro-spin columns from 200 mL of K2EDTA samples of patients’ population to investigate the possible use of plasma cfDNA as a feasible and reliable biomarker to describe/monitor the severity of IE and TI complications.

E1577
LEFT VENTRICULAR HYPERTRABECULATION BY CARDIAC MAGNETIC RESONANCE IN THALASSEMIA INTERMEDIA PATIENTS: FREQUENCY AND PROGNOSTIC ROLE
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Background: Differentiation of left ventricle non-compaction (LVNC) from hypertrobrubeculated LV due to a negative heart remodeling in thalassemia intermedia (TI) can depends 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 lower specificity to identify the true LVNC in TI. Anyway, the Piga’s criterion could easily detect a negative heart remodeling in TI patients.

Aims: The aim of our study was to prospectively assess whether the Piga’s criterion had a prognostic role for adverse cardiovascular outcomes in TI patients.

Methods: We studied prospectively 168 TI patients (81 males, mean age 38.32 ±11.61 years) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Standard cine steady-state free precession sequences were acquired and used for the calculation of biventricular function parameters (short-axis) and for the calculation of the thickness of the non-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=7.19, 95% CI=2.02-25.51; P=0.002) and cardiac complications (HR=3.66, 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).

Summary/Conclusions: Based on our data a NC/C ratio >2.5 provides prognostic information for patients with TI about the risk of developing cardiac complications.

E1578
NITRIC OXIDE DYSREGULATION IN BETA-THALASSEMA MAJOR: RELATION TO PULMONARY HYPERTENSION
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Background: Pulmonary hypertension (PH) is emerging as one of the most devastating complications of beta-thalassemia major. Chronic hemolysis and iron overload constitute a major source of strong oxidative stress. Free heme radicals and red cell membrane elements resulting from hemolysis have a negative effect on the intrinsic nitric oxide (NO) production and arginine availabil-
ability. Deficiency of both biochemical mediators promotes vascular injury in the pulmonary vasculature resulting in further endothelial dysfunction, with subsequent intensified reduction of nitric oxide. The role of nitric oxide dysregulation is well-studied in non-transfusion dependent thalassemias and in sickle cell disease, but yet not very well-characterized in beta thalassemia major.

Aims: The aim of our work is to study the relation between intrinsic nitric oxide level and the evolution of pulmonary hypertension in beta thalassemia major. Methods: This is a case-control study, including all patients with beta thalassemia major above 12 years of age, undergoing follow up in pediatric hematology unit and in medical research institute, university of Alexandria, Egypt throughout a period of 6 months from 1st of July till 31st of December 2016. All patients were screened for pulmonary hypertension by echocardiography, and those who have high tricuspid regurgitant jet velocity (TRV≥2.5m/sec.) underwent cardiac catheterization.

Results: The present study included 52 thalassemic patients, 28 males and 24 females. Their 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 pathophysiological 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 THALASSEMAIA MAJOR PATIENTS

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Background: The new parameters of cardiac function, derived from two-dimentional 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) and to assess its specificity and sensitivity in comparison with cardiac MRI T2*

Methods: This cross sectional study included 30 transfusion dependant β-thalassemia patients aged between 11–20 years recruited from the Pediatric Hematology&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 Tracking),MRI T2* were done.Cardiac affection by speckled was defined as decreased longitudinal strain less than 11 percentage or affection of any segment less than 11 percentage.

Results: Cardiac affection by speckled echocardiography was found in 10 patients(33.3%), 8 of them (80%) had normal ejection fraction and normal shortening fraction, while 9 had iron overload by Cardiac MRI T2*. Patients with mean serum ferritin ≥140mg/dL were followed for 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 pathophysiological effects of nitric oxide deficiency in beta thalassemia major e.g. its relation to coagulopathy and platelet aggregation are recommended.

E1581

EFFICACY, SAFETY AND GENETIC BASIS OF VARIABILITY OF RESPONSE TO HYDROXYUREA THERAPY IN BETA THALASSEMAIA: 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 synthesized γ-chains which neutralize the excess α-chains and therefore improves symptoms.

Aims: Systematic review of literature to evaluate the efficacy, safety and the genetic basis of variability of response to hydroxyurea therapy in beta-thalassemia patients.

Methods: Research sources used were: MEDLINE (PubMed), EMBASE (Ovid) and Cochrane from June 1993 till June 2016. Eligible articles were reviewed and data including patients’ characteristics, duration of treatment, outcome, toxicity and impact of genetic mutation on response to hydroxyurea therapy was extracted. Major respondents were those who became transfusion independent after hydroxyurea treatment, partial respondents had significant decline in transfusion requirements, poor respondents did not respond to hydroxyurea therapy. Statistical analysis software package 16 was used for data analysis.

Table 1.

<table>
<thead>
<tr>
<th>Type of Beta Thalassemia</th>
<th>Major Response</th>
<th>Partial Response</th>
<th>Poor Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-thalassemia major</td>
<td>30% (32%)</td>
<td>35% (35%)</td>
<td>15% (15%)</td>
</tr>
<tr>
<td>β-thalassemia intermedia</td>
<td>50% (50%)</td>
<td>20% (20%)</td>
<td>30% (30%)</td>
</tr>
</tbody>
</table>

Results: Thirty eligible studies comprising of a total of 1822 patients with beta thalassemia were identified. Of these (n=9, 30%) evaluated the direct effect of hydroxyurea therapy on beta thalassemia major patients, (n=11, 36%) evaluated beta thalassemia intermedia patients while (n=10, 34%) included both beta thalassemia major and thalassemia intermedia patients. Mean age of patients was 13.5 years. Mean duration of hydroxyurea therapy was 3.4 years. The mean increase in β-thalassemia was 10mg/g per day (8-15mg/kg). Table I showing number and percentage of patients having major, partial and poor response to hydroxyurea therapy. Only (n=12, 36%) studies evaluated the role of underlying genetic mutation on hydroxyurea response, out of these (n=6, 50%) studies found no significant correlation while (n=6, 50%) showed a positive correlation between common genetic mutations and hydroxyurea response. Hydroxyurea was found to be well tolerated, only (n=09, 01%) had transient myelosuppression.

Summary/Conclusions: Hydroxyurea is an effective and well-tolerated agent in the management of β-thalassemia (both intermedia and major). It reduces blood transfusion requirements either partially or completely in majority of patients. No significant correlation between response to therapy and underlying genetic mutation was found. More studies are required to fully establish the association of genetic mutation to drug response.

E1582

EVALUATION OF CONTINUOUS BLOOD GLUCOSE MONITORING MONITOR FOR DETECTION OF ALTERATIONS IN GLUCOSE HOMEOSTASIS IN BETA-THALASSEMIA PATIENTS

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Background: Glucose metabolism disturbances, among other endopathies, are a common feature of β-thalassaemia major (β-TM). Pancreatic iron overload and diabetes mellitus (DM) are common in β-TM patients. However, the relationship between iron stores and glucose disturbances is not well defined. Continuous glucose monitoring system (CGMS) enables more diagnostic accuracy and a better achievement of an optimal glycemic control. Aims: To assess the pattern of glucose homeostasis in patients with β-TM and detect early impairment in glucose metabolism and prediabetic state in β-thalassemia patients comparing oral glucose tolerance test (OGTT) and CGM system.

Methods: This cross sectional study was conducted on 200 patients β-TM patients. Patients were studied focusing on transfusion history, transfusion index, iron chelation therapy and compliance to chelation. Complete blood picture, markers of hemolysis, serum ferritin and random blood glucose (RBG) were measured. Patients with RBG ≥140mg/dL were subjected to OGTT, insertion of CGMS for 3 days, measurement of fasting C peptide, and serum insulin with calculation of HOMA-IR and assessment of HbA1c.

Results: Screening with RBG revealed that 20 patients (10%) had RBG ≥140mg/dL. Using OGTT, 7 (3.5%) patients were in the diabetic range, 7 (3.5%) patients were in the prediabetic range, 7 (3.5%) patients in the impaired glucose tolerance range, 7 (3.5%) patients in the impaired fasting glucose range and 7 (3.5%) patients had normal glucose tolerance. Using CGM, 22 patients (11%) had impaire glucose tolerance.

Discussion: CGM is useful in the primary and secondary prevention of diabetes in β-TM patients. Further studies are required to fully establish the association of genetic mutation to drug response.
The percentage of diabetic patients diagnosed by CGMS was significantly lower 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 were significantly higher among patients with RBG ≥140mg/dL (p=0.001). It was noted that 65% of RBG ≥140mg/dL were noncompliant and 75% of patients on desferrioxamine therapy had RBGE ≥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 RBG. As regard to 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 (prediabetes) among patients with β-TM appears to be promising and superior to other known diagnostic modalities namely OGTT and HbA1c.

E1585
ASSOCIATION OF SP1 POLYMORPHISM IN THE COLLAGEN TYPE 1 ALPHA-1 (COL1A1) GENE WITH OSTEOPOROSIS IN CHILDREN WITH BETA-TALASSEMIA
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Background: Osteoporosis is a progressive bone disease that is characterised by a decrease in bone mass and density that leads to an increased risk of fracture. Early detection of mutation at the Sp1-binding site on the COL1A1 gene is mandatory in order to initiate preventive therapy before the occurrence of fracture in childhood.

Aims: To study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with Beta thalassemia.

Methods: A prospective case control study was carried out in the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals on forty thalassemic patients (21 females &19 males) aged 6-18 years during their regular follow-up visits (22 patients with thalassemia major and 18 with thalassemia intermedia)and forty age- and sex-matched healthy children as a control group. All patients and control were subjected to full medical history, thorough clinical examination and laboratory investigations in the form of complete blood count, Hb electrophoresis, Calcium level Serum ,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 genotype there was high percentage of heterozygous Ss (G/T) and homozygous ss (T/T) genotype in beta thalassemia major 55.63%, 13.67% than thalassemia intermedia 50.6%, 0%, respectively. There was significant relation between COL1A1 genotypes and Calcium level (p=0.02). But there was no significant relation between COL1A1 genotypes and osteocalcin, alkaline phosphatase levels and DEXA among studied groups.

Summary/Conclusions: SP1 polymorphism in collagen gene could be of clinical value in identifying the thalassemic patients at risk of developing osteoporosis.

E1586
UNUSUAL MOLECULAR MECHANISMS IN THE ORIGIN OF ALPHA-THALASSEMIA
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Summary/Conclusions: We demonstrated that serum GDF-15 levels were increased in thalassemia major patients. GDF-15 levels is correlated with hepatic iron overload but not cardiac iron overload. It may be due to lower number of thalassemic patients with abnormal cardiac T2* MRI. GDF-15 may be a valuable parameter to assess iron overload in thalassemia major, but further studies are needed.

E1584
THE IMPORTANCE OF SERUM GDF-15 LEVELS TO ASSESS IRON OVERLOAD IN PATIENTS WITH THALASSEMIA MAJOR
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Background: There is growing interest in noninvasive assessment of iron accumulation in patients with thalassemia major. Magnetic resonance imaging (MRI) have become widely available in recent times.

Aims: We aimed to evaluate the importance of serum GDF-15 levels for monitoring the iron overload in patients with beta thalassemia major.

Methods: Forty-six patients aged between 1 and 25 years were included in the study. Serum levels of GDF-15, ferritin, troponin, AST and ALT were studied. T2 MRI was performed for all patients. The relationship between GDF-15 hormone levels and T2 MRI, ferritin levels, sex, annual transfusion volume, splenectomy was evaluated.

Results: Of 46 patients, 20 were male (%43.5) and 26 were female (%56.5), with a median age of 12.4 years. Mean serum ferritin level was 2752, 1523±105.78 ng/ml. Mean GDF-15 level was 9672,757±7590,36pg/ml. Mean duration of T2 MRI was 32.50±11.33 ms for heart and 4.97±4.78 ms for liver. 12 patients were underwent splenectomy. Serum GDF 15 levels were significantly higher in thalassemia major patients than in normal levels. According to T2 MRI levels, serum GDF-15 levels were significantly higher in patients with hepatic iron overload. There was a negative correlation between hepatic T2*MR values and serum GDF-15 levels. However, there was not significant correlation between cardiac T2*MR and serum GDF-15 levels. Splenectomy had no effect on GDF-15 levels (Table 1).

Table 1.
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 HBβ: IVS1-6 (C>T) is the most frequent genotype of beta thalassemia intermediate in our population and was even termed “beta thalassemia intermediate type Portuguese” (Tamagnini et al, 1983). The IVSI-6 (C>T) carriers (heterozygous) are characterized by mild hypochromia and microcytosis, with a moderately increased in HbA2, that may be even less than 3.5%. The correct identification of these carriers is important, especially when facing a couple who intends to have children.

Aims: To evaluate the percentage of individuals with hypochromia and microcytosis and Hb A2 between 3.2% and 3.4%, who are beta thalassemia carriers, alerting for the need to adapt the cut-offs of HbA2 values to the genetic background of different populations.

Methods: Methodology used in our laboratory from January 2007 to January 2016. The inclusion criteria were the simultaneous presence of hypochromia and microcytosis, with normal or slightly elevated RDW, without sideropenia, with HbA2 between 3.2-3.4%, should be screened for mutations in the HBβ gene, in order to rule out beta thalassemia carriers due to Beta+ mutations. As HBβ IVSI-6 (C>T) mutation is one of the most frequent beta thalassemias mutations in Portugal, and in Mediterranean basin, it is necessary to consider the screening and the identification of the carrier state. The classic rule of HbA2> 3.5% for the diagnosis of beta thalassemia minor may underestimate this pathology and lead to an incorrect genetic counseling.

Results: Respect for the inclusion and exclusion criteria we have identified 43 individuals with hypochromic and microcytic anemia, HbA2 ≥3.2% and ≤3.4%, in which the HBB gene mutations were screened. Among the 43 subjects, nineteen presented HbA2≥3.2% (19/43), eleven HbA2=3.3% (11/43) and thirteen had HbA2=3.4% (13/43). The IVSI-6 (T>C) mutation was identified in 2 subjects with HbA2=3.2% (10%), 5 with HbA2=3.3% (45%) and 7 with HbA2=3.4% (54%). No other HBB gene mutations were detected. The remaining individuals who revealed to be a very unusual case of acquired alpha-thalassemia associated with a myelodysplastic syndrome.

Summary/Conclusions: We have found five distinct deletions and one indel, all in heterozygosity. The deletions range from approximately 3.3 to 323 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 which presented the 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 deletions to provide patients with appropriate genetic counseling.

Table 1.
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 cross-clamping the hepatic artery and portal vein with a microvascular atrumatic clip for 90 min. Then a clip was taken off to provoke the reperfusion of hepatic blood flow, which was monitored on the surface of left lateral lobe by Laser Doppler flowmetry (ALF21, Advance Co, Tokyo, Japan). The hepatic blood flow was measured again 24 h after reperfusion and mice were then sacriﬁced. The liver weight and histological analysis of the liver tissue (haematoxylin and eosin staining) were performed for all groups.

Results: The liver weight 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 signiﬁcantly reduced 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 ADAMTS 13 TESTING IN STROKE EVALUATION: A REVIEW OF 619 CONSECUTIVE YOUNG STROKE AND TIA PATIENTS

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Background: Young ischaemic stroke patients undergo extensive investigations yet around 40% remain of undetermined cause. Complex and costly thrombophilia testing is routinely sent despite limited evidence linking to arterial disease. A full blood count may be ignored but is potentially more helpful in suggesting myeloproliferative disease or thrombotic thrombocytopenic purpura (TTP) as cause.

Aims: 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. We also performed a mutational analysis for TTP.

Methods: We retrospectively reviewed consecutive clinical and laboratory records for all stroke and TIA patients <60 years presenting to a regional hyperacute stroke unit and daily TIA clinic from January 1st 2015- August 7th 2016. All those with thrombocytosis (defined as platelet count >400x10^9/L) and/or raised haematocrit (defined as Hct >0.45) were reviewed to see if a cause could be determined, and if not, whether JAK II analysis was considered and tested. We similarly examined patients presenting with thrombocytopenia (defined as platelet count <150x10^9/L) and, if no cause determined, whether ADAMTS13 testing was contemplated.

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, 18 (3.1%) thrombocytosis, and 26 (4.2%) thrombocytopenia. Of these, 7 patients demonstrated abnormalities of both cell lines. Of these initial 161 abnormal results, 119 (73.9%) were repeated but 42 (26.1%) were not. JAK II testing was deemed warranted in 17 (2.8%): a persistently raised or progressively raised haematocrit or platelet count respectively, with normal liver and renal function and no other explicable cause. JAK II mutational analysis was performed in 3 patients (0.5%). One was proven positive for the V617F mutation, hence diagnosed with polycythemia vera. Of the 2 negative JAK II results, one patient was subsequently diagnosed with chronic myeloid leukaemia. 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 TTP 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 TTP. In the other patient, ADAMTS13 was <5%, confirming TTP and facilitating life-saving plasma exchange in place.

Summary/Conclusions: In stroke patients <60 years, one quarter had abnor- malities 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. In addition, a 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.
PLINK software was used to determine the allelic frequencies, concordance with Hardy Weinberg equilibrium and association between risk alleles and 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 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 FXI rs2036914 (C) and rs2289252 (T), respectively, and 0.295 and 0.417 for ABO rs2519093 (T) and rs8176719 (C), respectively. The genotypes’ distributions were in agreement with the Hardy Weinberg equilibrium (p>0.05) for all SNPs. The risk 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 associated even after adjusting for age and sex (P<0.019 and p=0.005, respectively). ABO rs2519093 did not reach significant association with VTE in our population sample (p=0.184) as well as FXI 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: X²=58.5, p=0.015, OR=2.31; and 0 vs 2 or more risk alleles: X²=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.

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 3.3 years, with the greatest proportion of cases in the infant age group (52%, in the infant age category (3 months - 1 year)), while children above 1 year comprised 37% and neonates (<1 month) formed 8.6% of our sample. Patients were mostly born at full term (71.4%), although 45.7% of the neonatal and infant cases were premature. Catheter-related (CVC-VTE) comprised 55.7% of VTE cases. On the other hand, non-catheter-related (NCR) diagnoses were made in 19.2% of cases in 35.5% of patients with extremely 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 trigeminal pain, such as infection (12 cases), and use of asparaginase (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. About 30% of patients 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: The risk of 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.
E1596 DESCRIPTION OF THROMBOTIC EVENTS AND/OR PREGNANCY LOSS IN A COHORT OF HOMOZYGOUS CARRIERS FOR THE C46T POLYMORPHISM IN THE F12 GENE
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Background: The intrinsic pathway of coagulation is initiated by a serine protease named factor XII (FXII) in a reaction involving the contact system and triggers fibrin formation through activation of factor XI. In vitro, FXII triggers activation of the classic complement pathway and initiates the fibrinolytic system via plasma kallikrein mediated urokinase activation, whereas in vivo its role in the prothrombotic process is poorly understood. Factor XII (FXII) polymorphism in the 5’ untranslated region of the F12 gene (F12 C46T) is associated with lower levels of FXII. Its frequency varies widely across populations and ethnic groups, ranging from 0.18 in the Spanish population to 0.67 among Japanese. Homozygosity for the C46T polymorphism of the F12 gene has been proved to be an independent risk factor for thrombosis and unexplained recent spontaneous abortion. However, the precise role of this polymorphism as a thrombotic risk factor is controversial, and the evidence for an association between F12 C46T, venous thromboembolism (VTE) and myocardial infarction is weak.

Aims: To describe the occurrence of thrombotic events and/or pregnancy losses and the existence of other risk factors for thrombosis in a cohort of homozygous individuals for F12 C46T.

Methods: We retrospectively analyzed all the homozygous F12 C46T cases diagnosed in our laboratory from January 2015 to January 2017. Allelic discrimination using qPCR 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 or venous 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% asatic, 4.1% other. Decreased factor XII plasma levels were seen in 81, 42% of them, with mean factor XII levels 53.73% (27.5-107.5). Overall, 34.48% of the subjects had at least one thrombotic event. Type of thrombosis: 64.4% VTE and 35.6% arterial thrombosis. One (26.7%) or more than one (46.7%) additional thrombotic risk factors were found in patients with any thrombotic event. Presence of one or more CVRF was found in 66.7%. Familiar history of thrombosis was found in 16%, whereas 13% had a recent or active malignant neoplasm. Among women, 28.57% and 12.98% had one and more than one pregnancy loss respectively. Additional thrombotic risk factors were found in 60% of women with recurrent losses. One (43%) or more than one (34.3%) 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 post patients with a thrombotic episode had one or more additional risk factors. Nevertheless, up to 26.7% presented no other risk factor than homozygous F12 C46T, suggesting a relevant role in the pathogenesis of thrombosis. According to our results, the risk of abortion could be increased by the presence of homozygosity for F12 C46T, since it was the only thrombotic risk factor found in women with recurrent abortions. Further studies are needed to clarify the real contribution of F12 C46T to thrombosis and pregnancy losses on prospectively selected patients.

E1597 ANALYSIS OF CHRACTERISTICS 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 identifying those patients with hospital-associated thrombosis (HAT), defined as acquired thrombosis/ fibrinolytic activity within the hospital walls. In order to determine factors that may be contributing to HAT we have undertaken a root cause analysis (RCA) of those HAT cases deemed preventable. Of 394 HAT cases with sufficient data, 80 (20.3%) had a preventable cause, 27 receiving insufficient TP, 9 receiving delayed TP, 26 having no TP given though indicated and 18 not having a VTE risk assessment. Some cases of insufficient TP were deemed possible due to lowning standard patient >90kg. Of these HAT cases deemed unpreventable, 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 by current techniques. Key errors inapprciated 80s are failure to perform a timely VTE risk assessment and action with appropriate thrombo-prophylaxis. Full integration of electronic patient records with electronic precribing modules may reduce further these errors.

E1598 THROMBOSIS DURING INFANCY AND NEWBORN PERIOD: AN UNSOLVED 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, overactivity of procoagulants and deficiency/ dysfunction of fibrinolysis 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 newborns (<1-12months) and newborns(>12month) 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 we analyzed the thrombotic risk factors.

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 in 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 and 11 venous thrombosis (deep veins(4), renal veins(3), portal veins(3) cerebral vein(3)) were noted. In 2(9%) children birth thrombosis was diagnosed on the first day of life and 11 out of 22 patient had underlying risk conditions such as prematurity(3), perinatal hypoxia(2), necrotizing enterocolitis(1), congenital cardiac disorders(3),congenital nephrotic syndrome(1) and adrenal insufficiency(1).Moreover 6 out of these 22 thrombotic event catheter insertion was the associated risk factor and 4/22 had infection. Factor V Leiden mutation was found to be homozygous in 1/18 and normal in 17/18. Heterozygous prothrombin 20210A mutation were detected in 1 out of 18 and homozygous MTHFR C677T mutation was found in 3/13 patient. Half of them (12/54) were initially treated with LMWH and TPA were used as a thrombotic event. In 5 case we used warfarin for the first time. During the follow up period 1 patient had an amputation, 5 patient deceased; one because of sepsis and the rest 4 had primary disease and thrombosis. The site of location in 55 thrombotic events during the infancy period involved deep venous thrombosis (22), cerebral sinusovenous thrombosis (10),cardiac(8), portal(3), renal(1) veins and cerebral arterial (7),femoral arterial(3),abdominal aortic thrombosis(1).In this group 42(76%) out of 55 had an underlying disorder and most common associated risk factor for this age group was inserted catheter related thrombosis, infection and surgical operations.Initial treatment choice was LMWH in 25(45%) and during the follow up 10 had been changed to enoxaparin. In 21 resolved, 10 had parsial thrombosis, 4 deceased and 10 loss to follow up.

Summary/Conclusions: During the first month of life thrombolic complications is 40 times higher than at any other pediatric age. As previously reported venous thrombosis which mainly affect the limbs, the right atrium and renal veins are more frequently seen than arterial thrombosis in newborn infants with a male predominancy is compatible with our findings. In the absence of randomized clinical trials the choice of anticoagulation and the duration of treatment for this age group is still controversy beside the complex mechanism and a high mortality& morbidity rate. Although clinical and laboratory data of neonates were compatible with infants, treatment choices differ between these two groups and it sems that thrombolytic treatment was tend to be used more commonly in the neonates without any complication.

E1599 THE QUALITY COMPOSITION OF SOLUBLE FIBRIN MONOMER COMPLEX FRACTION FOR ACUTE AND POST ACUTE ISCHEMIC STROKE PATIENTS
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Background: During the first month of life thrombotic complications are more frequent than at other pediatric ages. As previously reported venous thrombosis which mainly affect the limbs, the right atrium and renal veins are more frequently seen than arterial thrombosis in newborn infants with a male predominancy is compatible with our findings. In the absence of randomized clinical trials the choice of anticoagulation and the duration of treatment for this age group is still controversy beside the complex mechanism and a high mortality& morbidity rate. Although clinical and laboratory data of neonates were compatible with infants, treatment choices differ between these two groups and it seems that thrombolytic treatment was tend to be used more commonly in the neonates without any complication.
and the reading was done independently by two different technicians or biologists 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 pathologic 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 cardiacembolic 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 0,2 ml was applied on Healthcare Biochemistry, Educational and Scientific Centre “Institute Of biology and medicine”, Kyiv, Ukraine.

**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 proteins, even a year after stroke soluble fibrin monomer complex content was higher compared 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.

**Summary/Conclusions:** It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

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**Figure 1.**

**Evaluation of a Rapid Nanoparticle-Based Lateral Flow Immunoassay (Stic Expert HIT) for the Diagnosis of Heparin-Induced Thrombocytopenia in a Cardihothoracic Hospital**

**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 IgG, 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 methods 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 (Stic Expert HIT) was performed on plasma from 35 patients from July 2016 until January 2017 and the reading was done independently by two different technicians or biologists.

**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 doubtful 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 positive 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. Nevertheless all 13 patients were found not to suffer from the HIT syndrome with the ‘4Ts’ scoring system.

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**E1601**

**Audit of ‘Door To Needle’ Time in Administration of Prothrombin Complex Concentrate to Patients Requiring Urgent Reversal of Anticoagulation**

**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 (Beprix®).

**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 33%. The remaining indications (25%) were pre-urgent procedure/surgery, and soft tissue haematomata. 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.

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**E1602**

**The Importance of Platelet Membrane Fluidity and Oxidative Stress in Thrombotic Complications Acquired by Chronic Myeloproliferative Neoplasms Patients**

**Background:** Patients with chronic myeloproliferative neoplasms (MPNs) and chronic myeloid leukemia (CML) have a variety of functional and structural abnormalities of platelets. Many of them have thrombotic or hemorrhage complications. Platelet function is influenced by changes in membrane fluidity 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 Coletina Clinical Hospital Bucharest. The determination of platelet membrane fluidity was performed by fluorescence anisotropy measurements using as marker 1-(4-trimethylammoniumphenyl)-8-phenyl-1,3,5-hexatriene p-toluenesulfonate (TMA-DPH). We analyzed the fluorescence anisotropy of platelet membrane and correlate the result of a
different kind of treatment. Production of ROS was examined using fluores-
cent methods with DCFDA and Fluorolog spectrophotometer. Platelet receptor
expression was analyzed by flowcytometric method studying adhesion marker
(CD 42 and CD 42b) and aggregation marker (CD61, CD41).
Results: Patients with MPN and JAK2 mutation present a high level of flu-
orescence anisotropy than the JAK-negative group. Median value for JAK2 pos-
tive MPN group 147.2% CI for median value (157.7-150.6) vs JAK2 negative
MPN group 130.8% (124.6-138.3) p<0.001. There are no differences between CML
and MPN group. Our results confirm that fluorescence anisotropy is influenced
by medication taken. MPN patients who have taken Hydroxyurea alone had a
high-level 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, have a low level of fluorescence
anisotropy, median value and 95% CI for Hydroxyurea group 151 (137.1-158.6)
vs TKI group value 138 (124.4-147.8) p=0.04. No differences of fluorescence
anisotropy was observed between the group of MPN patients who received JAK
inhibitor (Jakavi) or Hydroxyurea The CD42b expression is low in patients versus
controls (median: 17.87% vs 94.16%, P<0.001), there is no difference in the
CD42a value range (P<0.51). The CD56/CD41 expression (GBP 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 MPN
and CML patients compared with healthy controls. CML patients in accelerate 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 modification 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 hos-
pitals and cancer treatment. However, CVC-related Venous thromboem-
bolemia (VTE) and iatrogenic pancreatitis are still occur. In the last years,
in pediatric age group the VTE is become an important and dangerous prob-
lem. In pediatric age group exact risk factors for CVC related venous throm-
bosism are showed in Table 1. In 27.5% (n=28) PE diagnosis preceded to
cancer diagnosis, in 26.5% (n=27) PE occurred at least 1 month beyond the
end of antineoplastic treatment and in 46.1% (n=47) PE was diagnosed during
the treatment (chemotherapy +/- radiotherapy). In this last group the median
time from the treatment beginning and EP diagnosis was 3 months (0-46). The
stratification according to the Khorana score (at baseline) was: ‘low risk’ (0 points)
intermediate risk’ (1-2 points) or ‘high risk’ (≥3 points) for VTE.
Methods: We analyzed the demographic characteristic, the Khorana score and
the antineoplastic treatment of oncologic patients diagnosed of pulmonary embolism (PE) from December 2010 until December 2016 at the Complejo Hos-
pitalario de Navarra. At baseline, the Khorana score classified patients as ‘low
risk’ (0 points) intermede risk’ (1-2 points) or ‘high risk’ (≥3 points) for VTE.
Results: 102 oncologic patients were diagnosed of PE. Patient baseline char-
acteristics are showed in Table 1. In 27.5% (n=28) PE diagnosis preceded to
cancer diagnosis, in 26.5% (n=27) PE occurred at least 1 month beyond the
end of antineoplastic treatment and in 46.1% (n=47) PE was diagnosed during
the treatment (chemotherapy +/- radiotherapy). In this last group the median
time from the treatment beginning and EP diagnosis was 3 months (0-46). The
stratification according to the Khorana score (at baseline) was: ‘low risk’ 21.3%,
intermediate risk 61.7%, and high risk 17%. In the intermediate risk group
(n=29) the drug-based therapy was: 44.8% platin (n=13), 6.9% gencitabine
(n=2), 2.5% lenalodamide (n=1) and 48.3% non-related-thermotherbolic treat-
ment (n=14). Most of cases (97.1%) were managed with LMWH (enoxaparin
1mg/kg/twice a day). Only 2 patients were treated with non-fractionated heparin
and 1, enrolled in a clinical trial, was treated with direct oral anticoagulants.
Summary/Conclusions: Nearly 2/3 of Khorana intermediate risk patients
developed a PE while on antineoplastic treatment and inside this group over
50% were treated with well-recognized high thrombotic-risk drugs. The inclusion
of antineoplastic drugs in a predictive thromboembolic model in oncologic
patients could improve the benefit-risk of the use of LMWH prophylaxis in some
patients without a high risk Khorana score but however at high risk of thrombosis.
More prospective studies are needed to analyse the benefit of antithrombotic
prophylaxis in oncologic patients receiving outpatient chemotherapy treatment.
**Transfusion medicine**

**E1605**

**CLINICAL OUTCOMES AND UTILIZATION OF BLOOD BANK RESOURCES OF PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP), HEMOLYTIC UREMIC SYNDROME (HUS), AND OTHER MICROANGIOPATHIC HAEMOLYTIC ANAEMIA (MAHA)**

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**Background:** TTP, HUS and other thrombotic microangiopathy are rare, complex clinical syndromes which are characterized by thrombotic microangiopathy, microangiopathic haemolytic anaemia, (MAHA) and systemic thrombosis. The introduction of plasma exchange (PEX) has dramatically reduced the mortality of these patients, and has become standard of treatment. Although the clinical outcome of these conditions is heterogeneous, 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 Throm Haemost 2017)

**Results:** In our series, the causes and number (%) of MAHA were: TTP-HUS (18, 42.9%), autoimmune disorder-associated MAHA (13, 31% i.e. 9 SLE and 4 Sjögren’s syndrome), cancer-related MAHA (4, 9.5%), drug-induced (3, 7.1%), post-transplant and infection-related microangiopathy (4, 9.5%). The average number of PEX sessions required to achieve overall clinical response in TTP, autoimmune-associated MAHA, HUS and drug-induced microangiopathy was 18.2±17.9, 11.5±7.6, 13.0±8.7 and 7.3±6.7, respectively. The mean follow up time was 40.8 months. 5 patients (11.6%) died during the course of treatment in index hospitalization, 12 (27.9%) were refractory to PEX and 24 patients (55.8%) responded to PEX, and 1 patient was lost to follow up.

**Discussion/Conclusions:** Treatment of MAHA includes PEX and standard of care was 59% and 80% (p=0.51), 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 Vincristine (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). SD20±5
dx20. The overall mean EX index was 12.3±1.4 and the patient index was 10.1±1.3. Besides, 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.

**Summary/Conclusions:** The clinical outcome in terms of survival in our cohort is in keeping with that of other registry and cohort (Hovinga et al Blood 2010). Our data which demonstrate the health care resource utilization show that management of these patients is expensive. While small in terms of incidence, it poses an economic burden disproportionate to its overall size.

**E1606**

**HEPATITIS E VIRUS: INVESTIGATION IN NORTH ITALIAN BLOOD DONORS**

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**Background:** Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide. It is a food-borne pathogen that to transfusion safely. Recent data from Europe showed a HEV IgG prevalences of 6.8% in German blood donors, 27% in Dutch blood donors, and 52% in an hyperendemic area in the South of France.

**Aims:** The aim of this study was to determine the prevalence of anti-HEV reactivity and HEV viremia in Italian blood donors, in order to estimate the risk of transmission.

**Methods:** Nearly 10,000 samples were collected from anonymized, unpaid donors at the “Lecco processing and validation blood center” (Lombardy, Italy) from June to July 2016. Samples were tested individually (individual-donation nucleic acid test [ID-NAT]) for HEV RNA using the Procleix HEV assay (95% limit of detection 7.9 IU/mL). Initial TMA-reactive samples were retested and considered positive if the retest result was reactive. For the serology study, a subset of 2000 donations was tested for HEV IgG using DiaPro HEV ELISA kit (Diagnostic BioprobesSrl, Milano, Italy). HEV IgG and IgM were analyzed in ID-NAT positive samples at the time of donation and in the follow up, collected one year after the index donation.

**Results:** The prevalence of IgG anti-HEV in north Italian blood donors was 7.4%. Nine out of 9,726 donor samples gave reactive values by the ID-NAT assay for HEV RNA. Among them, only one sample was confirmed to be reactive in additional TMA tests. None of the 9 HEV RNA initially reactive samples had circulating IgM or IgG antibodies against HEV, suggesting that only the repetitive reactive donor showed a IgM and IgG seroconversion, indicating primary HEV infection. Therefore, we estimated that the risk of receiving a potentially infectious blood unit is of 1:10,000 (upper bound of the 95% confidence interval, 1:1700).

**Summary/Conclusions:** Our data which demonstrate the health care resource utilization show that management of blood donors, and 52% in an hyperendemic area in the South of France.

**E1607**

**SHORT-TERM ADMINISTRATION OF RECOMBINANT HUMAN ERYPOIEIN DECREASES B CELL IN HUMAN PERIPHERAL BLOOD**

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**Background:** Erythropoietin (EPO) is hematopoietic factors participating in red blood cell production, and accelerates proliferation and inhibits apoptosis of erythroblasts. It is reported that EPO has pleiotropic effects including anti-apoptotic action for some cells, antioxidant action, vasculization action, and promoting the multiplication and development of the bone marrow. There are conflicting results of small cohorts as to its effect on blood immune cells.

**Aims:** We analyzed peripheral white blood cell subsets in patients who received one bolus administration of recombinant human erythropoietin (HuEPO) to examine the effect of EPO on human immune system.

**Methods:** One hundred nineteen autologous blood donors (male/female 62/57) in Gunma University Hospital were enrolled in this study after written informed consent. All the patients had no infections or inflammation. Forty nine patients were treated with rHuEPO (Epoeitin alpha or Epoeitin beta (24,000 IU, respectively)) once after blood donation because of low hemoglobin concentration and 70 were not treated. Peripheral blood samples were obtained at the time of the first phlebotomy and after 1 week from the same patient. We measured the number of WBC, lymphocytes, myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), CD4+ T cells, CD8+ T cells, Natural killer (NK) cells, B cells, monocytes, and neutrophils of peripheral blood before and after HuEPO administration by flow cytometry. Absolute numbers and percentage of lymphocytes in WBC decreased significantly after rHuEPO administration from 1885.0±520.8/µl to 1798.7±439.0/µl, in absolute number (p<0.019), and from 33.2±8.57% to 30.0±7.32% in percentage (p=0.023). The numbers of whole WBC, mDC, pDC, monocyte and neutrophil did not change significantly. In respect of lymphocyte subsets, absolute number of CD8+ T cell, NK cell and B cell significantly decreased from 358.9±257.0/µl to 311.5±210.9/µl (p=0.019), and from 298.3±192.4/µl to 239.9±158.2/µl (p<0.01), respectively. Regarding B cell subset, absolute number of naive B cell and IgD-CD27+B cell significantly decreased from 171.3±93.5 /µl to 153.0±84.2 /µl (p<0.01), respectively. Regarding B cell subset, absolute number of naive B cell and IgD-CD27+B cell significantly decreased from 171.3±93.5 /µl to 153.0±84.2 /µl (p<0.01), and from 16.5±13.6 /µl to 12.9±12.7 /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 naive B cell and IgD-CD27+B cell in total B cell did not change. These suggested that whole B cell decreased, not a specific subset of B cell. In non treatment group, there was no change of percentage of cell subsets.

**Summary/Conclusions:** These findings suggested that just one administration of rHuEPO influenced human immune system, especially via reduction of B cell in peripheral blood, with unknown mechanism so far.
Background: At most centers, the majority of patients who request bloodless medicine are members of the Jehovah’s Witness (JW) faith. But, there are no standard, established guidelines to manage pancytopenia in these patients, nor are there many studies to inform optimal treatment approaches. The most troublesome patients who request bloodless medicines are patients with hematologic malignancy: The treatments of these patients are considerable challenges. They have not only problems of severe pancytopenia, but also require intensive chemotherapy. Since 2000, our hospital has been a bloodless center. This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soonchunhyang university hospital.

Methods: A retrospective review of medical records was performed for 44 patients with hematologic malignancies and aplastic anemia who request bloodless medicine from January 2006 to December 2015 at Soonchunhyang university hospital.

Results: Of 44 patients, 48% were men (n=21) and 52% were women (n=23). The median age of the study population at the time of diagnosis was 62 years (range 18-87). Thirteen patients (29.5%) were acute leukemia, 15 (34.1%) patients with non-Hodgkin’s lymphoma (NHL), 2 (4.5%) patients with aplastic anemia (AA), 6 (13.6%) patients with chronic myeloid leukemia (CML), 4 (9%) patients with myelodysplastic syndrome (MDS) and 4 (9%) patients with multiple myeloma (MM). Thirty one patients were treated with chemotherapies and 13 patients were treated with supportive care only. Among 44 patients 27 patients were died. Most common cause of attribution to death was anemia (92.5%). And Chief complaint at death was dyspnea (88%). Median survival of acute leukemia was 1 month (95% CI, 0.41-1.59).

Table 1. Summary/Conclusions: In bloodless treatment, CML, MM and lymphoma had a relatively good prognosis. However, AML and MDS were showed a poor prognosis. Therefore, further studies are needed to improve survival for bloodless patients with hematologic malignancies.

E1609

PREOPERATIVE ANEMIA: A SINGLE INSTITUTION EXPERIENCE IN SPAIN

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Background: Preoperative anemia is considered as a strong predictor of postoperative red cell transfusions, and has also been linked to increased morbidity and mortality in surgical patients, but it is frequently overlooked.

Aims: The objective of this study is measure of real impact of preoperative hematological assessment and optimization of anemic patients in terms of decreasing blood cells transfusions.

Methods: 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 eliology. The primary outcome was the response to therapy defined as reaching the Hb level >13 gr.dl increasing of >2 gr.dl from basal level, and the rate of blood transfusion.

Results: Mean age was 70.4 years, with a male-female ratio of 1:2, and the patients were divided into 2 groups according to the bleeding risk: high risk 74% (hip and knee replacement, cysterectomy, colostomy, maxilofacial surgery), and low risk 26% (gastroenterology, urology 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%), while 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 anaplasia, the type of transfusion and the disease, with myeloid malignancies requiring more transfusions. The establishment of haplo-identical transplantations has increased the transfusion needs due to longer period of aplasia.
Acute lymphoblastic leukemia - Biology

PB1611
BOTANICAL ALKYL HYDROQUINONE HQ17(3) EXERTS CYTOTOXICITY TO T(9;22) PHILADELPHIA CHROMOSOME SUP-B15 ALL CELLS THROUGH INDUCING ENDOPLASMATIC RETICULUM STRESS, AUTOPHAGY, AND AIF TRANSLLOCATION

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Background: Patients suffering from Acute lymphoblastic leukemias (ALLs) harboring t(9:22) genetic abnormality are classified very high risk (VHR) ALLs displaying poor clinical outcome irrespective of intensive chemotherapies and tyrosine kinase inhibitor treatment. Development of new adjuvant therapeutic will have potential as adjuvants controlling the VHR-Ph+-ALL cells refractory to therapy-induced cytotoxicity, 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 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 necrosis.

Aims: To investigate the characters of, and the molecular pathways involved in the HQ17(3)-induced non-classical death on VHR-ALL cells, and help develop the therapeutic strategies for the VHR-ALLs.

Methods: Cell growth inhibition in response to HQ17(3) 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 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 aggregation of ectopically expressed EGFP-LC3. Western blot analysis were used to p-eIF2a, ER chaperone Grp78, spliced RIP1-mediated controlled necrosis.

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 catalytic release of lysosomal membrane permeabilization (LMP) as cathepsin inhibitors did not attenuate the cell death. HQ17(3) enhanced autophagy as revealed by aggregation of ectopically expressed GFP-LC3. Inhibition of autophagy by Bafilomycin A1 or knockdown of the essential autophagy-related Beclin1 by shRNA could partially attenuate HQ17(3)-induced cell death. Further, HQ17(3) treatment gave rise to early ER stress as revealed by enhancement of eIF2a phosphorylation and up-regulation of ER chaperone Grp78. HQ17(3) induced nuclear translocation of AIF, in compatible with mitochondria disturbance and caspase-independent cell death thereafter.

Conclusion: 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 leukemia (aALL) patients, by applying targeted next generation sequencing (NGS) on MiSeq System. We analyzed 34 de novo ALL patients (17 cALL and 17 aALL) using TruSeq Amplicon – Cancer Panel (TASCAP) that targets mutational hotspots in 48 cancer related genes (212 ampiclons). 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 2800 x per million. Ten genes were discarded due to insufficient coverage, therefore we analyzed a total of 183 ampiclons from 38 genes. Variants were identified in relation to the GRCh37 reference genome by applying a Bayesian approach and compared to previously found somatic genetic variants in leukemias and lymphomas.

Results: We identified a total of 331 (159 cALL, 172 aALL) variants in the codon 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: 10-14). Overall, 79% 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: 3, range: 1-5). Moreover, we identified 5 NFM mutations in STK11 gene, 3 in ABL1, RET KRAS and 2 in HNF1A, NRAS, and NOTCH1. Observed in individual patients detected mutations predominantly disrupted Ras/RTK pathway stack. As STK11, KIT, MET, NRAS, KRAS, PTEN). Additionally, we identified 5 patients with the same mutation in HNF1-A gene coding for transcriptional factor, disrupting both Wnt and Notch signaling pathways. Notch pathway was disrupted in two patients in which detected variants affected NOTCH1 gene. HNF1A and NOTCH1 variants were mutually exclusive, while genes involved in Ras/RTK pathway exhibit a tendency of mutation accumulation.

Conclusion: Our targeted NGS study showed low number of recurrent somatic mutations among both childhood and adult ALL patients. RNA sequencing followed by single-nucleotide signal analysis and targeted sequencing in some patients revealed new key signaling pathways, primarily Ras/RTK 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.

PB1612
RELAPSED LOW HYPODIPLOID ACUTE LYMPHOMATIC LEUKAEMIA IN A LI-FRAUMENI PATIENT

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Background: A 7-year-old girl presented with backache, leg pain and difficulty walking. She was referred to the local paediatric oncology service, as she was known with Li-Fraumeni Syndrome (LFS). Cancer spectrum in LFS includes soft tissue sarcomas, osteosarcoma, breast cancer, brain cancer, leukaemia and adrenocortical carcinoma. The patient’s mother had breast cancer twice, and both her (monogygotic) twin sister and older sister had adrenal cortical carcinomas removed. The patient was born with left sided twinning defects (absent left kidney, malrotation, absent left ear and ear canal, Arnold-Chiari malformation with spinal cord syrinx and hydrocephalus). At presentation there was a suspicion of a disseminated malignancy. She underwent an MRI scan which showed extensive changes in her spinal column and hips. Bone marrow biopsy revealed acute lymphoblastic leukaemia (ALL). Paucity of diagnostic material restricted the genetic analyses. G-banding showed 46.XX[14], with FISH demonstrating loss of FOXO1/13q14.11 [44%], gain of MYC/8q24.21 [9%], ETVS-RUNX1 NEGATIVE, gain of RUNX1/21q22.12 [21%] and BCR-ABL1 NEGATIVE. The patient was treated according to NCI-103 Risk criteria. The least intensive regimen of the UK ALL 2003 trial. She achieved morphological remission after a 3-drug induction, and successfully completed further treatment, including intensification/CNS directed phase, interim maintenance and 2 delayed intensification blocks. She completed 5 years of follow-up and was transferred to the Long Term Follow-Up Clinic, when she presented with hypercalcaemia. Peripheral blood and bone marrow biopsy confirmed a diagnosis of ALL. Although the phenotype resembled the profile of the first presentation of leukaemia, the genetic aberrations appeared incongruent.

Aims: Establish the origin of the second episode of ALL in a patient with known Li-Fraumeni Syndrome. As treatment and outcome for relapsed ALL in comparison with a secondary, primary ALL are completely different, this information was critical to guide further management.

Methods: We set out to comprehensively characterise the second ALL, including conventional G-banding and fluorescence in situ hybridisation (FISH). The
acquired results were compared with those derived from the first ALL diagnosis.

Results: The median age was 36.4 [range: 2-97] years. The CD4+/CD8+ ratio showed a prominent difference with a higher CD4+/CD8+ ratio than the non-leukemic group (27.1% [IQR 21.6-33.2] 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=0.69). 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 PD1-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 groups of patients, p16INK4a deletion was associated with poor event-free survival. The prognostic impact of CDKN2A/p16INK4a deletion in adult ALL patients appears 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 - bipheno
typal ALL. Interphase fluorescence in situ hybridization (FISH) was performed for detection CDKN2A deletion, TEL/AML1, MLL rearrangement, MYC (8q24.21) translocation, TP53 deletion, IAMP21.

Figure 1.
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 biphenotypal 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 data 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=0.003), with high 9p21 (37/39) expression (the median is 3092 E/L, p=0.0004) and no associating with CR and relapse incidence was found. We didn’t revealed relationship between CDKN2A deletion and ALL, TEL/AML1 rearrangement, MYC translocation, TP53 mutation and IAM21. CDKN2A deletion didn’t have statistically significant impact on outcome of patients. The five-year OS of patients with and without deletion was 85% and 76% (p=0.35); DFS was 92% and 65% (p=0.07), 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 association between deletion of the CDKN2A gene and with known cytogenetic prognostic factors. However patients with T-cell ALL and CDKN2A deletion had a more favorable 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). Additionally, we set up a genomic qPCR to screen for CDKN2A and CDKN2B deletions for patients enrolled in the protocol RALL-09. We calculated CNA values for the normal cells (2N) contaminant present in the diagnostic samples. In addition, the results obtained by the array and/or qPCR were checked by FISH, when samples were available. Cumulative incidence of relapse (CIR) and OS were analyzed after censoring the patients at the time of all-cause death. All statistical analyses were performed using the R software (version 3.5.1).

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 B to 28% (15/53) of the samples. Global alterations in CDKN2A/B locus were observed in 9 (29%) of the patients. Microdeletions obtained from the qPCR corroborate 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 of CDKN2A/B CNA status in 49 cases with adequate follow-up. Median age (range) was 34 (16-68) years, 76% males, median WBC count 34 (0-431.0) x10^9/L. Immunophenotype: pro-T+pre-T (n=21), cortical T (n=21), mature T (n=7). CR was achieved in 92% (45/49) and MRD levels <0.1% at the end of induction were attained in 81% of patients. A trend for better OS was observed for patients with heterozygous or homozygous deletion of CDKN2B (61% [40%-82%]) vs non deleted patients (25% [0%-54%], p=0.028), whereas no clinical impact was observed for the CNA status in the CDKN2A gene. No influence of CDKN2A or CDKN2B CNA status on CIR was observed. By multivariate analysis only the MRD level at the end of induction influenced on OS (p=0.028, HR=5.58 [2.1; 25.76]) and on CIR (p=0.07, HR=3.67 [9.0-15.63]).

Summary/Conclusions: 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.

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PB1617

BUTEIN KILLS ACUTE LYMPHOBlastic LEUKEMIC CELLS IN VITRO AND IN VIVO THROUGH FOXO3A AND CASPASE-DEPENDENT APOPTOTIC PATHWAYS

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Background: Acute lymphoblastic leukemia (ALL) is a common hematological malignancy in children. Discovering and developing effective chemotherapeutic drugs are needed for ALL.

Aims: In this study, the anti-leukemic effect and the potential molecular mechanisms of butein on ALL were investigated.

Methods: We examined the rate of apoptosis of CEM-C7 (T-ALL), CEM-C1 (T-ALL), MOLT-4 (B-ALL), RS4-11 (B-ALL) cell lines and primary ALL blasts isolated from acute lymphoblastic leukemia (ALL) patients. We tested the expression of the caspase-9, poly ADP-ribose polymerase (PARP), nuclear forkhead class box O3a (FOXO3a) and BCL-2 interacting mediator of cell death (BIM) using western blot assay. We also set up the xenograft murine model to examine the anti-leukemic effect of butein in vivo.

Results: We tested that 10μM of butein was found to significantly induce the cellular apoptosis of ALL cell lines and primary ALL blasts in a dose-dependent manner. We also established the cleavage of caspase-9 and PARP. We also found that butein stimulated FOXO3a localization, enhanced the binding of FOXO3a on the BIM gene promoter and then increased the expression of BIM. Moreover, we showed that FOXO3a knockdown significantly decreased the apoptosis by butein, whereas overexpression of FOXO3a enhanced the butein-induced apoptosis. However, overexpression of FOXO3a mutation (C-terminally truncated FOXO3a DNA-binding domain) decreased the apoptosis by butein through decreasing the expression of BIM. Furthermore, treatment with butein was highly efficacious in vivo, with enhanced reduction of tumor burden in a xenograft model of ALL.

Summary/Conclusions: Our results therefore demonstrate the therapeutic potential of butein for ALL via FOXO3a and caspase-dependent apoptotic pathways.

PB1618

GENOMIC LANDSCAPE AT DIAGNOSIS AND RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Childhood acute lymphoblastic leukemia (ALL) is the most common of pediatric malignancies, but intensive chemotherapy now allows to obtain complete remission in over 90% of the cases. Nevertheless, 1 out of 5 children will 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 collected at diagnosis and relapse. They were analyzed by high density 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: We identified new methylated loci in the EGIS classification between diagnosis and relapse. Diagnostic cytogenetics prognosis was good for 3 children, poor for 3 (AmpAML1, KMT2A and complex cytoready) 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 presented 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 t(4;8) translocation was found to be more complex with 7 and 8 CNA on chromosomes 4 and 8. Patients with the most CNA and LOH also had a complex karyotype. Anomalies were observed in hot spot regions in 8p (comprising CSDKNA2/2B, PAX5 and JAK2) for 5 patients and 12p (including ET6V) for 3. Stable CNA were observed in the JAK/STAT pathway in 2 patients (JAK2) and LOH in the RAS/RAF pathway (NRAS) in 1. Using the genetic classification of Moorman et al based on SNP array for 8 genes at diagnosis (IKZF1, CSDKNA2/2B, PAR 1, BTG1, EBF1, PAX5, ET6V and RB1), SNP reclassified our patients in 3 of good prognosis and 5 of poor prognosis, with a median of 2 CNA for the 8 genes of interest. The 2 patients with cytogenetic intermediate prognosis would thus probably have been considered for a more intense therapy regimen, i.e. allogeneic stem-cell transplantation. Moreover, SNP showed that 2 patients acquired an IKZF1 deletion, also of poor prognosis, while none of the children had TP53 mutation at diagnosis nor relapse.

Summary/Conclusions: SNP array allowed to identify additional anomalies (compared to karyotype) in all children tested and changed the prognostic value of diagnostic anomalies. Moreover, the identification of anomalies in the JAK/STAT pathway could indicate a treatment by tyrosine kinase inhibitors, which would possibly have positively modified outcome. Taken together, this new technology combined with classical analyses at diagnosis might modify therapeutic options in childhood ALL, especially in the subgroup with a normal karyotype.

PB1619
SCREENING OF NUDT15 GENE VARIANTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: In cells, while DNA bases can be protected by double helix formation and nucleosome packaging, deoxyribonucleotides triphosphates are 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 thiopeurine metabolism. Thioquinones are active metabolites of thiopeurines. Mechanisms of action of thioquinones are disruption of DNA synthesis and induction of apoptosis. NUDT15 inhibits incorrect base pairing and apoptosis through catalysis of thioquinone hydrolysis. Tanaka et al. claimed that, besides TPMT variants in Japanese patients, there might be possible additional factors that may influence thiopeurine 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 diagnosed with Pre-B ALL at Lôsete Hospital. DNA samples were isolated by using 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 rs3831098 (c.158+52_158+53insGGGGGCGTCCGCAAGGGGCACTCC). The other intronic variation was defined as rs79687000 (c.158+117C>T). rs3831098 was determined in one of the 83 patients and rs7968700 was found in three out of the 83 patients (Table 1).

Table 1.

Summary/Conclusions: The changes in NUDT15 that we found have not been previously reported in pediatric ALL patients. We do not know if these changes have an effect on pre-mRNA or "splice" regions and ALL. This issue needs further investigations in a large number of children with leukemia. We are planning the screening of other exons of NUDT15 in order to evaluate for possible applications to clinical practice (e.g. cytopenia).
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 might be inferior, but remain largely elusive.

Aims: This study aimed to determine engraftment and growing ability of primary adult ALL samples in immunodeficient mice. Genetic engineering was performed to evaluate transduction efficiencies by lentiviruses in PDX ALL cells.

Methods: Primary adult ALL and AML material was transplanted into NSG mice in the absence of total body irradiation. Both frozen as well as fresh patient material was used. Human CD45 and human CD38 were stained in blood to analyze engraftment and growing ability. Lentivirus transduction was performed using lentiviral vector systems and monitored by expression of fluorescent markers and flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of fluorescent markers and flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of fluorescent markers.

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 of 5 weeks compared to fresh samples which could already be isolated 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 MLL-AF4 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 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 ALL 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 LYMPHOBLASTIC LEUKAEMIA
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Background: Epigallocatechin-3-gallate (EGCG) and menadione (vitamin K3, MD) are known as potent apoptogens in cellular models for acute lymphoblastic leukemia (ALL) – Jurkat T cells.

Aims: The goal of this study was to explore the chemotherapeutic potential of MD combined with EGCG or DOX, and to determine whether there is a synergistic interaction between these agents that could significantly enhance their antitumoral effect in a cellular model of ALL. We investigated the antiproliferative effect of MD combined with EGCG or DOX respectively on human leukemia Jurkat lymphoblasts. Some underlying cellular mechanisms were also scrutinized.

Methods: Cell suspensions of Jurkat lymphoblasts were treated at various concentrations of EGCG, 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 spectrophotometry, using the fluorescent probes CM-H2DCFDA and JC-1, respectively.

Results: In the monotherapy, clonogenic survival (C20) ranged from 117 µM mean 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). EGCG induced a depolarizing effect at the mitochondrial level, most likely via interference with the opening of the mitochondrial permeability transition pore. The combination EGCG:MD induced cell cycle arrest in G2/M and S phases in a synergic manner (Δψm) (Pearson correlation coefficient r= −0.100) or between [Ca2+]m and reactive oxygen species (r=0.437). EGCG and MD interact with the second specific target of DOX: mitochondrial DNA. EGCG may increase mitochondrial membrane stability, enhancing the antiproliferative effect of DOX most likely by increasing the affinity of DOX for nuclear DNA.

Summary/Conclusions: Our results support the notion that the combinations EGCG: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 LYMPHOBLASTIC LEUKAEMIA
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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 high 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].

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 and ERG. PCR (Tie-probe primer HS0307979_g1) was tested carried deletions targeting ERG exon 5. Results of DUX4 qRT-PCR were plotted against transcriptional target of DUX4.

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 qRT-PCR (Tie-probe primer HS0307979_g1) were surprisingly inconsistent with Western blot analysis - which could only in part be explained by DUX4 being a one exon gene. NALM-6 was the only cell line expressing the DUX4 protein. Likewise, 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-IGH translocation, expresses the DUX4 protein and an ERG mRNA variant including the alternative exon 6. ERG deletions were present in cell line SUP-B15. DUX4 and ERG expression in pre-B-ALL cell lines were not detected.

References:

PB1624
NATURAL HISTORY OF SECONDARY MULTIPLE PROLIFERATION WITH MONOSOMY 7 FOLLOWING TREATMENT OF RELAPSING ACUTE LYMPHOBLASTIC LEUKAEMIA
J. Buls1, A. Pobudejska-Pieniazek1, L. Sędzek2, A. Lopeż2, M. Jara-Acevedo3, A. Kowalska-Pawlak4, A. Sonsala1, A. Orfao3, T. Szczepański1
1Department of Pediatric Hematology and Oncology, Zubrza, Medical University of Silesia, Katowice, Poland, 2Department of Hematology, University of Silesia, Katowice, Poland, 3Cancer Research Centre (IBMCC, USAL-CSIC), Institute for Biomedical Research of Salamanca (IBSAL); Department of Medicine and Cytometry Service (Nucleus Research Laboratory, 1Department of Microbiology and Immunology, University of Silesia, Katowice, Poland, 4Department of Microbiology and Immunology, University of Silesia, Katowice, Poland

Background: Approximately 90% of children with acute lymphoblastic leukemia (ALL) are cured with current treatment protocols. However, 15-20% of the patients still experience disease relapse. Most of these patients develop secondary therapy-related leukemia or myelodysplasia.

Aims: We present a case of a 11-year-old boy with the history of relapsed ALL treated with different number of regimens. 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 and ERG. PCR (Tie-probe primer HS0307979_g1) was tested carried deletions targeting ERG exon 5. Results of DUX4 qRT-PCR (Tie-probe primer HS0307979_g1) were surprisingly inconsistent with Western blot analysis - which could only in part be explained by DUX4 being a one exon gene. NALM-6 was the only cell line expressing the DUX4 protein. Likewise, the alternative ERG transcript with alternative exon 6 was observed in NALM-6 only.

Summary/Conclusions: In conclusion, 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:
PB1625
IDENTIFICATION OF KEY GENES AND CONSTRUCTION OF MICRONA–MRNA REGULATORY NETWORKS IN MULTIPLE MYELOMA BY INTEGRATED MULTIPLE GEO DATASETS USING BIOINFORMATICS ANALYSIS
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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 first CR (n=23) and at relapse (n=6). The expression level of mRNA encoding L-cellular Fas-associated death domain-like interleukin-1β converting enzyme inhibitory protein (c-FLIP) was assessed by real-time PCR. Changes in the expression level of HDAC before and after chidamide treatment were also assessed by western blot. Necrosis and apoptosis after chidamide treatment were assessed by flow cytometry. Changes in expression level of c-FLIP, protein before and after treatment were assessed by western blot. Expression level of early apoptotic protein, key proteins of necrotic process were assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis were assessed by western blot. The regulating effect of chidamide on downstream genes of NF-κB pathway including cyclinD1, TNFα, IL-2, IL-8 were assessed by RT-PCR.

Results: The expression level of c-FLIP, 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, mRNA is associated with patient risk stratification, white blood cell count at initial presentation, serum lactate dehydrogenase (LDH), serum level of hydroxybutyrate dehydrogenase (HBDH), CD45, HLA-DR, SIL-TAL1 fusion gene and complex karyotype, and is not associated with age, sex, plasma fibrinogen level, and the chromosomal aberration 6q-. Patients who did not achieve CR during first chemotherapy had a higher c-FLIP, mRNA level than those who did (p<0.05). The expression level of histone deacetylase (HDAC) is higher in deranged mononuclear cells of T-ALL patients, Jurkat and HUT-78 cell lines. After treatment with chidamide, the expression level of histone deacetylase was significantly decreased in both cell lines. Chidamide induced necrosis and apoptosis in Jurkat and HUT-78 cell lines. After apoptosis inhibitor was applied, chidamide primarily exert its effect of inducing cell death by inducing necrosis. Chidamide inhibits the transduction and translation to c-FLIP L, gene. When apoptosis is inhibited, chidamide upregulates the expression level of receptor-interacting protein 3 (RIP3) and the phosphorylation level of mixed lineage kinase domain-like (MLKL). After treatment with chidamide, the phosphorylation level of c-FLIP L and 6q- positive was both significantly decreased.

Summary/Conclusions: 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 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 could be used as a prognostic marker in T-ALL. Chidamide suppresses histrone deacetlyation in Jurkat and HUT-78 cell lines. Chidamide induces necrosis in Jurkat and HUT-78 cell lines by down regulating the transcription and translation of c-FLIP L gene. Chidamide induces necrosis in Jurkat and HUT-78 cell lines via the classical NF-κB signaling pathway.

PB1626
CYP1A1 AND CXCL12 GENE POLYMORPHISMS IN CHILDHOOD ACUTE LYMPHOBLASTIC 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. Despite high survival rates (higher than 80%), a significant number of children relapse and for them the outcome remains poor. Epidemiological studies that examined possible risk factors of acute leukemias, 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 important role in tumor growth and invasion. The polymorphism rs1801157 of the CXCL12 gene has been investigated concerning the disease pathogenesis. Moreover, CYP1A1 gene belongs to family 1, subfamily 1A1 of cytochrome P450. CYP1A1 protein is a phase I xenobiotically metabolizing enzyme that activates the conversion of environmental chemicals into carcinogens. The above gene contains two important single nucleotide polymorphisms, CYP1A1*2A (rs4646803) and CYP1A1*2C (rs1048943), which are associated with an increased risk of leukemia.

Aims: The study of single nucleotide polymorphisms rs1801157 of CXCL12 and CYP1A1*2C (rs1048943) in children with B-lineage ALL.

Methods: Thirty children with B-lineage ALL (19 boys, mean age 6.8 years) were included in this study; 11 children (age 5.1 years) were in the control group and 81.6%, 16.4% and 2.0% in adult control group respectively. In the CYP1A1 loci, the frequencies of AA, AG, and GG genotype are 13.3%, 86.7% and 0% in children with ALL, 90.0%, 10.0%, 0% in children control group and 81.6%, 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.
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 15-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 ALL with common phenotype with two populations; one being strong CD19+/CD66c- and one with dim CD19+/CD66c-. FISH showed >10 RUNX1 signals in clusters in 95% of cells, while 52% showed BCR-ABL1 positivity. Bone marrow karyotyping yielded metaphases of poor quality (Figure 1).

**Summary/Conclusions:** ALL with iAMP21 is a rare subtype with distinct clinicopathological characteristics. Presenting with only mildly elevated WBC in older children is typical, relapses are frequent if standard risk chemotherapy is administered. Association with BCR-ABL1 translocation is rare, having been reported so far 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.
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-medulillary relapse is a rare event occurring in only 8% of the patients, of whom only 1.4% present a skin relapse which harbor a dismal prognosis (Gokbuget et al.). Aims: Herein, we report the efficacy of Blinatumomab in two patients presenting with extra-medulillary relapse of ALL.

Methods: The first patient (a 40-year-old man) was diagnosed a CD19+ Ph - B-ALL in 2009. He received a chemotherapy regimen according to the GRAALL protocol (Huguet et al.) until complete remission (CR). In 2015, he 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 antitumor chemotherapy (COPRAALL 2007 regimen) (Domenach et al.), with no efficacy (cutaneous blast 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/t 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 CD3+ T cell infiltrate”. The prominent dermal CD3+ lymphocytic infiltrate suggests promising activity in extra-medulillary relapse. Further studies are required to confirm a Blinatumomab-based strategy in extra medulillary relapsed B-ALL. This may provide a better understanding of how cytolytic synapses between T lymphocytes and intradermal blasts happen and the underlying homing mechanisms involved.

**PB1629**

**COMPLETE REMISSION WITH BLINATUMOMAB IN TWO PATIENTS WITH SKIN RELAPSED B-CELL ACUTE LEUKEMIA**

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1CHU Bordeaux Haut léveque, pessac, France

**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 (4–106). There were 8, 14, and 5 patients with early MRDneg, late MRDneg, and MRDpos, respectively. There were no differences in patient characteristics by bone marrow status, except for the duration to achieving remission (Table 1). There were significant differences in the 3-year DFS rates among patients with early MRDneg, late MRDneg, and MRDpos (100% vs 72.9% vs 20%; p=0.001) (Figure 1). Patients undergoing transplantation had better prognosis than those receiving chemotherapy alone in the late MRDneg group (100% vs 40%; p=0.028), whereas there was no difference in the early MRDneg group (100% vs 100%; p=0.48).

**Table 1. Patient characteristics by MRD status as assessed with the modified BIOMED-2 PCR for IgH protocol.**

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<th>MRD Status</th>
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<tr>
<td>Early MRDneg</td>
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<tr>
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Figure 1. The status of minimal residual disease was associated with prognosis.

**Summary/Conclusions:** The modified BIOMED-2 PCR protocol is a highly accurate and reliable method of MRD assessment in adult ALL. It predicted treatment outcomes in adult Ph (-) ALL, and patients with late MRDneg might derive a high survival benefit from allogeneic transplantation. Finally, the accuracy and reliability of the modified BIOMED-2 PCR for IgH were confirmed with a comparison to quantitative real-time PCR for BCR-ABL using samples from patients with Philadelphia-positive ALL (data not shown).

**PB1631**

**SYSTEMATIC LITERATURE REVIEW OF PEGASPARAGFNE 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 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 (4–106). There were 8, 14, and 5 patients with early MRDneg, late MRDneg, and MRDpos, respectively. There were no differences in patient characteristics by bone marrow status, except for the duration to achieving remission (Table 1). There were significant differences in the 3-year DFS rates among patients with early MRDneg, late MRDneg, and MRDpos (100% vs 72.9% vs 20%; p=0.001) (Figure 1). Patients undergoing transplantation had better prognosis than those receiving chemotherapy alone in the late MRDneg group (100% vs 40%; p=0.028), whereas there was no difference in the early MRDneg group (100% vs 100%; p=0.48).

**Table 1. Patient characteristics by MRD status as assessed with the modified BIOMED-2 PCR for IgH protocol.**

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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).
an IV administration option, and improved immunogenicity. Clinical outcomes in the adult ALL population are less well understood.

**Aims:** To assess the relative clinical benefit of PEG-ASP vs native ASP in 1st line treatment in newly diagnosed adult ALL patients in terms of event-free survival (EFS) and overall survival (OS). Safety outcomes were also examined.

**Methods:** A systematic literature search was conducted using a standardized search algorithm within the National Library of Medicine PubMed database to identify available evidence for newly diagnosed patients treated with adult ALL protocols that use PEG-ASP or native ASP. Randomized, observational, and cohort studies were included, with the predefined clinical outcomes of event-free-survival (EFS) and overall survival (OS). Data was pooled with 95% confidence intervals (CIs) calculated using the logit transformation.

**Results:** A total of 30 studies were identified that met the pre-specified inclusion criteria, with 10 studies providing data for PEG-ASP and 23 studies for native ASP. The pooled estimate of 2-year EFS for adult ALL patients treated in 1st line with asparaginase was 48.0% (95% CI: [10.8; 85.2]) for PEG-ASP and 66.0% (95% CI: [52.0; 77.0]) for native ASP. Similarly, the pooled estimate of 5-year OS was 64.5% (95% CI: [61.5; 67.5%]) for PEG-ASP and 46.8% (95% CI: [33.6; 60.1]) for native ASP. In very high risk ALL patients, the pooled estimate of 5-year OS was 57.1% (95% CI: [52.4; 61.7%]) for PEG-ASP and 35.3% (95% CI: [21.7; 51.7]) for native ASP.

Findings for safety outcomes were consistent with product labeling for both asparaginas.

**Summary/Conclusions:** The systematic literature review highlights a positive clinical effectiveness profile for PEG-ASP in regards to EFS and OS in the treatment of newly diagnosed adult ALL patients with less frequent administration and similar safety profile as compared with native ASP.

**PB1632**

**A COMPREHENSIVE ANALYSIS OF PATIENT- AND THERAPY-RELATED FACTORS AFFECTING THE TOXICITY OF PEGYLATED-ASPARAGINASE FOR THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** The application of pediatric regimens in the treatment of adult acute lymphoblastic leukemia (ALL) has led to a significant improvement in patients outcome. However, concerns about the feasibility of more intensive therapies and of the use of pegylated L-Asparaginase (PEG-ASP) in adult patients have emerged. Some patient-related risk factors as high BMI or hepatic steatosis have been already identified as risk factors, but few data are available on the synergic toxic effect from other concomitant drugs.

**Aims:** The aim of this study was to evaluate the incidence of PEG-ASP related adverse events in a cohort of adult ALL patients in order to identify potential patient and therapy-related risk factors contributing to toxicity.

**Methods:** Since 2013, 21 adult ALL patients received PEG-ASP therapy in our institution. Median age was 44 (range 19-76): 12 patients were treated in front-line setting (7 according to a full pediatric protocol) whereas 9 patients received therapy for relapsed/refractory neoplasm. We retrospectively analyzed each single course which included PEG-ASP administration as an independent event, accounting 41 episodes. Patients’ features (age, BMI, disease status) and concomitant therapies were accurately analyzed as factors potentially affecting PEG-ASP toxicity. The incidence of major thrombotic/bleeding complications and grade III/IV hepatic or pancreatic toxicity was analyzed; toxicity grading and management of PEG-ASP related complications were performed according to guidelines recently published by Stock et al.

**Results:** No grade III/IV pancreatic, thrombotic or hemorrhagic adverse events were recorded. A total of 8 episodes of grade III/IV hepatic toxicities were observed. In 3 cases, grade IV toxicity was observed. Those patients experienced unexplained severe weight gain and painful epigastralgia, a common picture resembling sinusoidal occlusive disease, ultrasonography showed acute liver steatosis. All 3 patients received concomitant therapy with idarubicin, vincristine and vancomycin. In univariate analysis, the incidence of grade III/IV hepatic toxicity was significantly higher when concomitant chemotherapy with at least 2 mg/sqm cumulative dose of vincristine (p = 0.044, HR 4.75) or at least 16 mg/sqm cumulative dose of idarubicin (p = 0.046, HR 1.45) were administered.

**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.
Summary/Conclusions: The results of this study inform the magnitude of cost in Germany associated with adult rALL patients who or without an HSCT after relapse. The cost estimates provide a benchmark against which new treatment options for rALL can be compared. For future studies, it would be important to determine the magnitude of benefit such as long-term survival and other health consequences associated with HSCT as well.

PB1634
RETROSPECTIVE STUDY OF ADULT ALL IN MEXICO CITY: FIRST REPORT OF THE WORKING GROUP ON ACUTE LEUKEMIA
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Background: The prognosis of adult acute lymphoblastic leukemia (ALL) is dire, with a long-term survival of 40-50%. This disease entity is probably more frequent in the Latino population. Several studies have reported a worse prognosis in Hispanics with ALL as well as a greater incidence of the Ph-like genetic signature; however, the data is inconclusive in the Mexican population and there are no existing large multicenter series of ALL patients in Mexico that analyze survival.

Aims: The aim of this study was to describe the incidence, clinical and biological characteristics as well as the survival of ALL patients in 5 referral hospitals in Mexico City.

Methods: A working group known as the Grupo de Trabajo de Leucemia Aguda (GTLA), was created as a result of an initiative of the Mexican Group for the Study of Hematology (Agrupación Mexicana para el Estudio de la Hematología) to promote acute leukemia research in Mexico. This is the first report of the GTLA which includes 5 referral hospitals in Mexico City. A retrospective, multicenter, descriptive study of adult ALL patients treated between 2009 and 2015 was conducted.

Results: We included 559 adults in 5 centers in Mexico City. Their median age was 28 years (14-81): adolescents and young adults (AYA) 67.3%; adults 24.7% and elderly adults 8.1%. Tumor lysis syndrome was detected in 9.8% of all patients. Cytogenetic information was unavailable in 45% of cases due to lack of access or growth in metaphase. Among cases that could be analyzed, a normal karyotype was the most frequent (70.5%), followed by Ph+ (16.7%).

Patients were considered high-risk in 52.1% cases. The most frequently used drug protocol was Hyper-CVAD, in 47% of cases. Complete remission (CR) was achieved in 67.1% of patients, and 18% required a second cycle for CR, while 13% were primarily refractory. A mortality rate during induction was registered as 10.6%, and there were 11.4% deaths while in CR. Among patients in CR, 59.1% relapsed. At the time of analysis, 26.7% of patients were alive, with a median OS of 12.97 months and a DFS of 16 months. Only 5.7% were able to achieve a first CR and go to marrow transplantation (Allo-HCT). OS at 3 years was 22.1% and by age group: AYA 25.7%, adults 17.4% and elderly adults 0% (p=0.0001). On multivariate analysis, significant risk factors for OS were the age group, ECOG, the presence of the tumor lysis syndrome and liver function test abnormalities while protective factors included early CR and an Allo-HCT.

Summary/Conclusions: Outcomes are poor in adult ALL patients treated in these referral centers in Mexico City. This may be explained by the high incidence of AYA patients and the low frequency with which they are treated with regimens containing L-asparaginase. The incidence of the Philadelphia chromosome is lower than reported, which could be due to a real difference between populations or due to aspects related to cytogenetic techniques. Based on these results, the GTLA’s objectives will be: to standardize diagnostic testing evidence of AYA patients and the low frequency with which they are treated with allo-HCT in referral centers in Mexico City. This may be explained by the high incidence with which they are treated.

Summary/Conclusions: The frequency of IKZF1 gene deletions in patients 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 these deletions may be more prognostically valuable in patients with Ph-negative ALL.

Aims: To evaluate the frequency and prognostic impact of mutation status of IKZF1 in patients with de novo BCR-ABL1-negative and BCR-ABL1-positive B-cell acute lymphoblastic leukemia.

Methods: The study included adult patients (median age 27, range 17-56; 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 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) in BCR-ABL1- pos B-cell ALL patients with IKZF1 mutations and without was 37.5% and 57.1% (p=0.77), relapse - free survival (RFS) - 25% and 33.3% (p=0.88), respectively. In patients with BCR-ABL1- neg ALL the IKZF1 deletions were revealed in 8 (22%) of 36 patients (4 cases with del 4-7 (50%), 2 - del 2-7 (25%), 1 – del 2a-8 (12.5%) and 1 in patient all types of deletions were determined (del 7, del 4-7, del 2a-8)). The median follow-up time in 36 patients was 22 months (range: 0.5-84 month). 4 patients died of the disease (11%) and 2 of infections, 30 patients are alive. OS for patients with BCR-ABL1- neg ALL with IKZF1 mutations and without was 100% and 60.2% (p=0.77), RFS - 75% and 40.2% (p=0.74), respectively.

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.

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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**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-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 MRD after first induction was 74%, compared to 52% in MRD+ (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 intense 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**

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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, cortico-steroid 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 gene polymorphisms, which are important in bone mineral and matrix formation, have effects on bone turnover in patients with ALL.

**Methods:** Fifty children with ALL who were diagnosed and treated with BFM-95 protocol (25 girls, 25 boys) between 1998-2008 and 96 healthy children at Dokuz Eylül University Medical School were enrolled in this study. Polymorphisms of vitamin D receptor (VDR) Fok1 gene and the collagen Col1A1 gene were studied from peripheral blood samples of the patients that were collected before initiation of chemotherapy protocol. After genomic DNA extraction, VDR Fok1 gene and colloidal Col1A1 gene polymorphisms were analyzed by poly-merase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The data including age, sex, leukemia risk group, presence or absence of relapse were all noted. Bone marrow density and markers of bone metabolism including serum calcium, phosphorus, serum alkaline phosphatase, parathyroid hormone and 25-OH D vitamin levels were all screened before initiation of maintenance treatment.

**Results:** The distribution of Fok1 and Col1A1 gene polymorphisms was similar both in the patient group and healthy control group. The frequency of gene polymorphisms in the patient group were 8% F. 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 LYMPHOBластIC LEUKEMIA TREATED WITH PEDIATRIC PROTOCOL: MONOCENTRIC STUDY

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Background: Several retrospective studies have confirmed that adolescents and young adults (Aya) with acute lymphoblastic leukemia (ALL) treated with pediatric protocols have better outcomes than similarly aged patients treated with adult protocols.

Aims: We reported results and feasibility of a pediatric-based protocol (EORTC 58951) in adolescents and young adults.

Methods: From January 2000 to December 2015, 72 patients aged 16 to 30 years with newly diagnosed ALL were treated, in the department of clinical hematology of Hedi Chaker Hospital, according to the pediatric protocol EORTC 58951. Further leukemia characteristics (Sex, White Blood cell count, Blasts phenotype, Cyto genetic results), we studied the protocol results: response to induction, risk group of treatment failure and its stressful treatment, not to high: VHR, remission rate, death rate, relapse rate and 5 years survivals (over all OS and event free EFS).

Results: Seventy two Aya ALL were treated with the pediatric protocol. The patients were 45 males and 27 females (SR=1:66). A WBC>100 G/l was noted in 32%). At presentation, the 4 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 out of 72 patients from protocol were eligible for allogeneic stem cell transplantation (SCT), among them 15 patients had a familial donor and 10 patients were allograft (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 the high toxic mortality rate.

PB1640

DEPRESSION AND SELF-CONCEPTION IN CHILDREN WITH ALL-TREATMENT

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Background: The prevalence of depressive disorder in children with leukemia at the end of induction and at the end of consolidation treatment was significantly increased. Self-Concept Scales were found lower in these patients.

Results: The prevalence of depressive disorder in children with leukemia at the end of induction and at the end of consolidation treatment was significantly increased. Self-Concept Scales were found lower in these patients.

Summary/Conclusions: The children with ALL receive long course chemotherapy and become distanced from their family, school and milieu and as a result, these patients are vulnerable to psychological problems. They are more depressive and have lower self-conception comparing to healthy children. It is important to provide psychological support to these children in addition to their chemotherapy.

PB1641

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 psychiatric medications, in patients treated for childhood ALL. All patients were treated in a single institution and followed the same chemotherapeutic protocol, according to which, corticosteroids are administered initially during the “induction” phase and then in multiple subsequent pulses.

Results: Seventy patients (mean age:4.04 years old, range:1-16) were treated in our AYA department. From January 2000 to December 2015, 9 (12.8%) children (6 boys, 3 girls) of mean age 12.3 years old (range: 10-15) experienced psychiatric - neurological symptoms and/or mental disorders, which included major depressive disorder, withdrawal, first psychotic episode, disorientation, visual hallucinations, mood swings and behavioral outbursts. Twenty patients had symptoms of minor depression, 14 patients with intermediate risk ALL and 2 with the High Risk group experienced psychiatric disturbances during the induction phase, while treated with dexamethasone at 10 mg/m2 for 21 days. Two patients of the High Risk group presented with behavioral effects one during the second HR2 block. Patients who had symptoms of major depression were treated with either fluoxetine, or/and risperidone, or/ and escitalopram for a period of time ranging of 5 days to 6 months. One patient experienced a psychotic episode during induction (Prot.II,phase 2) with aggression and violence towards others and had to be treated immediately with intramuscularly haloperidol and diazepam. All of our patients are alive and in remission, 7 off therapy for a period of 3 years, and 2 receiving maintenance therapy. Statistical analysis showed that severe psychiatric disturbances were observed more frequently in older patients and they were more common with the administration of dexamethasone than with prednisolone.

Summary/Conclusions: Severe psychiatric disturbances are not infrequent in pediatric 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.

PB1642

INCIDENCE, SEVERITY AND RISK FACTORS FOR NEUROLOGIC COMPLICATIONS ASSOCIATED WITH L-ASPARAGINASE TREATMENT IN PAEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Combined chemotherapy increased over time cure rate in patients with acute lymphoblastic leukemia (ALL). Among other things, one of the direct adverse effects of chemotherapy is affecting hemostasis inducing thrombosis or bleeding. Hemostasis can also be affected indirectly by the antithrombin III, protein C, protein S and by inducing endothelial damage. Thrombotic events are more frequent in children with leukemia and either may be related with prothrombotic status of the patient.

Aims: To evaluate incidence and severity of thrombotic or bleeding events in paediatric patients with ALL during chemotherapy.

Methods: We considered all patients hospitalized for ALL in the Pediatrics
Department of Clinical Institute Fundeni during 2010-2017 and received chemotherapy according to protocol ALL BFM 1995 and ALL BFM 2002, established after framing in the risk group.

Results: Over a period of 8 years in our department 280 patients with ALL received L-asparaginase in the induction phase. Neurological manifestation suggestive for bleeding or thrombotic events occurred in 9/280 (3.21%) patients. 2 patients were treared according to protocol ALL BFM 1995 and 7 patients were treated according to protocol ALL BFM 2002. M/F ratio was 4/5. Patients had 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 MRI) 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 different risk factors, genetics, and improvement in diagnosis and treatment. Armenia is considered to be a more ethnic nation.

Aims: We aimed to quantify the incidence of and mortality from acute leukaemias 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 leukaemia, 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 Hema-
tology 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 leukaemia were identified, 174 (62.1%) were male. The overall age incidence of leukaemia was 3.4 per 100 000 children-years. The higher incidence rates were noted in 2007, 2012, 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). There are three registered regions in Armenia—Lori, Vayots Dzor and Tavush with higher incidence rate (respectively 2.4, 2.4 и 2.9). There are three registrated regions in Armenia—Lori, Vayots Dzor and Tavush with higher incidence rate (respectively 2.4, 2.4 и 2.9). There are three registrated regions in Armenia—Lori, Vayots Dzor and Tavush with higher incidence rate (respectively 2.4, 2.4 и 2.9).

Summary/Conclusions: Inclusion of various genetic and cytogenetic characteristics on the results of allogeneic hematopoietic stem cell transplantation, including haploidentical one at our University over 2008 to 2015. Twenty-one patients (12 females and 9 males aged from 3 months to 48 years; median 18.9 years) were examined.

PB1644
LONG-TERM SURVIVAL OUTCOMES OF ADULT PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOSTATIC LEUKEMIA PATIENTS TREATED WITH IMATINIB OR DASATINIB
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Background: Acute lymphoblastic leukemia (ALL) with positive Philadelphia chromosome (Ph+) is a unique subset of ALL with poor prognosis. Recent studies have demonstrated improved survival outcomes in adult patients with Ph+ ALL with the use of tyrosine kinase inhibitors (TKIs) along with chemotherapy. However, there are very few studies that describe the comparative effectiveness of various TKIs in this patient population.

Aims: To characterize long-term survival outcomes including leukemia-free survival (LFS) and overall survival (OS) for Ph+ adult ALL patients treated with imatinib versus dasatinib.

Methods: Retrospective chart review was conducted at our institution. Patients >= 18 years old and diagnosed with Ph+ ALL between 2002 and 2015 were included. Analysis was done by intent to treat for patients initiated with imatinib or dasatinib at the time of Ph+ diagnosis. The primary endpoints were 2-year LFS and OS and secondary endpoints were complete molecular response (CMR; BCR-ABL1/ABL1 ratio <0.01% by PCR) and major molecular response (MMR; BCR-ABL1/ABL1 ratio <0.1% by PCR).

Results: Among 46 patients with Ph+ ALL, 74% (n=34) were in imatinib group and 17% (n=8) in dasatinib group; 9% were treated with other or no TKI (1 ponatinib and 3 with 0 TKI). Thirty-eight percent (n=13) of patients in imatinib group and 13% (n=1) in dasatinib group switched to a different TKI due to adverse effects or failure to achieve remission. There was a trend towards increased 2-year LFS for patients on dasatinib (HR 0.40, 95% CI: 0.14-1.14, p=0.09) and no difference in 2-year mortality (HR 1.00 95% CI: 0.46-2.17, p=0.99). Molecular response data was available for 61% (n=28) of patients; 75% of imatinib group achieved CMR or MMR (65% CMR) compared to 76% of dasatinib group (63% CMR) (p=0.98) (Figure 1).

Figure 1.

Summary/Conclusions: In conclusion, dasatinib, compared to imatinib, in combination with chemotherapy, may prolong LFS in patients with Ph+ ALL and may be a suitable first-line agent. Large, randomized studies are needed to better define a detailed treatment protocol in this high-risk patient population.

PB1645
OUTCOME OF ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A MIXED COHORT OF PEDIATRIC AND ADULT PATIENTS WITH KMT2A-AFF1 ACUTE LYMPHOBlastic LEUKEMIA
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Background: Acute lymphoblastic leukemia (ALL) is the most common cancer in children. Childhood ALL who received allo-HSCT, KMT2A-AFF1

Aims: To evaluate the prognostic impact of the different clinical and cytogenetic characteristics on the results of allogeneic hematopoietic stem cell transplantation in KMT2A-AFF1 acute lymphoblastic leukemia patients.

Methods: Retrospective analysis of treatment results was performed for a mixed cohort of the patients with KMT2A-AFF1 ALL who received allo-HSCT, including haploidentical one at our University over 2008 to 2015. Twenty-one patients (12 females and 9 males aged from 3 months to 48 years; median 18.9 years) were examined.
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 abnormalities, 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-AFF1 ALL patients treated with HSCT. The good results of allo-HSCT can be obtained using a haploidentical transplantation from parents that removes the problem of searching the HLA-matched donors in the Registers and, therefore, greatly simplifies the treatment.

PB1646

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 observes in case of targeted therapy. Hair pigmentation is a potential biomarker for the effectiveness of TKI.

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 targeted tyrosine kinase inhibitors in National Research Center for Hematology. One pt (pt 1) with AML had been receiving chemotherapy (decitabine, cytarabine, idarubicin) with continuous treatment of sorafenib. Three pts with Ph+ ALL received TKIs. Two of them with T315I mutation (pts 2, 3) received ponatinib and multikinase inhibitor (sorafenib). One pt (pt 5) had been receiving chemotherapy (decitabine, cytarabine, idarubicin) with continuous treatment of sorafenib due to refractory disease on the first-line therapy (pt 5). One pt with B-ALL was treated by sorafenib due to refractory disease on the first-line therapy (pt 5). The therapy was restarted in all pts with temporarily discontinuation after skin lesions disappearing (Figure 1).

Summary/Conclusions: The study has shown the stage of disease at the moment of allo-HSCT to be independent prognostic factor in a mixed cohort of KMT2A-AFF1 ALL patients treated with HSCT. The good results of allo-HSCT can be obtained using a haploidentical transplantation from parents that removes the problem of searching the HLA-matched donors in the Registers and, therefore, greatly simplifies the treatment.

PB1647

CYTOKINE RELEASE SYNDROME DURING 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, as well, in acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma (NHL). The disease has been treated with local and systemic chemotherapy with different hematopoietic growth factor support. The management of CNS relapse is guided by age, clinical presentation, brain computed tomography, and cerebrospinal fluid (CSF) analysis. CNS relapse is believed to negatively impact survival. The role of intrathecal therapy (ITT) in children with CNS relapse is not well established.

Aims: To describe a case of a massive acute leukoencephalopathy after only one ITT, 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, bioumoral and ultrasound findings. The cerebrospinal fluid (CSF) analysis was negative for cytology, Mycobacterium tuberculosis, fungi, and viruses. The child showed severe headache followed by skin urticarial rash, high blood pressure and hallucinations, rapidly evolving in flaccid paralysis of lower extremities. A brain magnetic resonance (MRI) showed diffuse areas of hyperintensity of white matter, parieto-occipital and subcortical areas, cerebellar region, optic chiasm and brainstem in T2-Flair sequences; spinal cord showed massive edema, especially in lobar region. The MRI pattern was interpreted as diffuse grade IV leukoencephalopathy of probable toxic nature.

Results: Patient received hydratation, allopurinol, acetazolamide and prophylaxis of seizures with levetiracetam. After few hours from ITT, 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 magnetic resonance (MRI) showed diffuse areas of hyperintensity of white matter, parieto-occipital and subcortical areas, cerebellar region, optic chiasm and brainstem in T2-Flair sequences; spinal cord showed massive edema, especially in lobar region. The MRI pattern was interpreted as diffuse grade IV leukoencephalopathy of probable toxic nature. The child, 30 h after ITT, 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 spontaneous breathing. After waking up, the child showed rapid neurological amelioration; the patient received oral dexamethasone without molecular remission on dasatinib and nilotinib therapy, received second-generation TKI (bosutinib). One pt with B-ALL was treated by sorafenib due to refractory disease on the first-line therapy (pt 5). One pt with B-ALL was treated by sorafenib due to refractory disease on the first-line therapy (pt 5). The therapy was restarted in all pts with temporarily discontinuation after skin lesions disappearing (Figure 1).

Summary/Conclusions: The study has shown the stage of disease at the moment of allo-HSCT to be independent prognostic factor in a mixed cohort of KMT2A-AFF1 ALL patients treated with HSCT. The good results of allo-HSCT can be obtained using a haploidentical transplantation from parents that removes the problem of searching the HLA-matched donors in the Registers and, therefore, greatly simplifies the treatment.
SEVERE HYPOFIBRINOGENEMIA ASSOCIATED WITH IMATINIB AND PREDNISONE THERAPY IN PHILADELPHIA CHROMOSOME–POSITIVE ACUTE LYMPHOPROLIFERATIVE LEUKEMIA

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Background: Hypofibrinogenemia associated to acute lymphoblastic leukemia (ALL) is rare and usually due to L-asparaginase. Consumption coagulopathy or therapy-related hematotoxicity are other possible explanations. Severe hypofibrinogenemia, not linked to the causes listed, was rarely reported and a role of steroid therapy on fibrinogen metabolism was suggested.

Aims: Our aim was to identify 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 LAL201-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 /d from day 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 2x10^9/L (blasts 15%), 8x10^9/L (blasts 30%) and 18x10^9/L (blasts 61%), while platelet count was reduced in 2 cases (6x10^9/L and 65x10^9/L). Coagulation tests remained in a normal range; platelet counts showed a trend to normalization. Early clearance of peripheral blood blasts was observed and when hypofibrinogenemia appeared no blast cells were detectable. At the end of induction bone-marrow evaluation demonstrated the absence of BCR-ABL transcript by qualitative RT-PCR. There were no bleeding events and only one patient received a prophylactic transfusion of fresh-frozen plasma (10 ml/kg) for fibrinogen <50 mg/dl on two occasions. Normal fibrinogen levels (≥165 mg/dl) were recovered at the end of steroid therapy.

Summary/Conclusions: We observed severe hypofibrinogenemia in Ph+ ALL patients older than 65 years treated with imatinib and high-doses steroid, while normal fibrinogen levels were detected in younger Ph+ ALL during intensive chemotherapy plus imatinib. In our experience, hypofibrinogenemia was not associated to major bleeding events, although its clinical significance should be investigated in larger series. Fibrinogen may recognize multiple metabolic pathways, also unrelated to in vivo coagulation and fibrinolysis; the correspondence between steroid treatment and hypofibrinogenemia seems to suggest that glucocorticoids may alter some steps in fibrinogen kinetics and could be considered as a cause of acquired hypofibrinogenemia.

LATE EFFECTS OF CHEMORADIOThERAPY ON THE ENDOCRINE SYSTEM IN CHILDREN WITH ACUTE LYMPHOPROLIFERATIVE 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 hormone 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 >30 kg/m². Bone age delayed in two patients. Four patients had IGF-1 value below <-2SD and two patients had adequate levels of growth hormone. Tanner stage of the patients were appropriate for their ages except for one patient with hypergonadotrophic hipogonadism and one patient with pubertas precocix. Subclinical hypothyroidism was detected in two patients.

Summary/Conclusions: Significant late effects may develop over time in children treated for ALL. For this reason long-term follow-up of these children is necessary. Because of the awareness of the late effects the treatment modified to reduce the risk of the 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 Sequase 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%]; 11/51 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, SF3B1, EZH2 and SETBP1 in 4/231 (1.7%), 4/231 (1.7%), 1/231 (0.4%) and 1/231 (0.4%) samples, respectively (Figure 1).

Summary/Conclusions: The results of mutational analysis of large cohort of AML patients show high heterogeneity of detected mutations. Surprisingly we have detected high percentage of patients with mutations in gene NRAS. Together with sequencing results from the time of remission/relapse/resistance of the disease, the data will enable to get more complex view on the development of AML in time.

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PB1651

INHIBITION OF LIN28B IMPAIRS LEUKEMIA CELL GROWTH AND METABOLISM IN ACUTE MYELOID LEUKEMIA
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Background: Current conventional chemotherapy for acute myeloid leukemia (AML) can achieve remission in over 70% of patients, but a majority of them will relapse within 5 years despite continued treatment. 2. The relapse is postulated to be due to leukemia stem cells (LSCs), which is different from normal hematopoietic stem cells (HSCs). LIN28B is microRNA regulator and stem cell reprogramming factor. 3. Overexpression of LIN28B has been associated with advance 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 signaling 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 firstly 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. IGFBP2/1 was confirmed to be a novel downstream target of LIN28B via let-7 miRNA. 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 leukemia and provide a rationale to target this pathway as effective therapeutic strategy.

Figure 1. Distribution of gene mutations with VAF ≥2% 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.

Summary/Conclusions: The results of mutational analysis of large cohort of AML patients show high heterogeneity of detected mutations. Surprisingly we have detected high percentage of patients with mutations in gene NRAS. Together with sequencing results from the time of remission/relapse/resistance of the disease, the data will enable to get more complex view on the development of AML in time.

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PB1652

Evaluation of Minimal Residual Disease in NPM1-Mutated AML Patients
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Background: Minimal residual disease (MRD) tests provide early identification of hematologic relapse and timely management of AML patients. About 60% of adult normal karyotype AML has a mutation in exon 12 of NPM1 gene. This mutation is specific for malignant clone and potentially is a good marker of MRD.

Aims: The aim of the study was to analyze the usefulness of NPM1 as a marker for MRD quantification in AML during follow-up.

Methods: Retrospective study included 34 patients with mutated-NPM1 and treated with intensive chemotherapy (2009-2015). Bone marrow (188) and peripheral blood (277) samples were analyzed from complete remission (MRD NPM1 negative). NPM1 detection was performed by quantitative RT-PCR (Gorello el al. Leukemia 2006). Patients were considered positive when presented >1 NPM1 sample positive or/and one sample NPM1 >0.02%. Cox regression was used for univariate analysis.

Results: Patients were segregated in 2 groups: Relapse patients (Group 1: 32.2%, 11/34) and no relapse patients (Group 2: 67.6%, 23/34). Group 1 presented MRD NPM1 positive in 9/11 (82%) of patients, the time from NPM1 to relapse was 4.6 months (1.6-24). NPM1 mean was 1.7 (0.03-9). Group 2 presented MRD NPM1 negative (>0.02% y/ or 1 determination) in 21/23 (91%) patients. Univariate analysis was performed and our results show that age, leukocyte, LDH and MRD NPM1 are prognostic factors for cumulative incidence of relapse (Figure 1).

Figure 1.
Summary/Conclusions: NPM1 is a useful marker for MRD quantification in AML patients undergoing intensive therapy. NPM1's 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+K12a 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+K12a 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 cytara- bine) to treat CD34+/CD38–/CD123+K12a 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 cytara- bine) in CD34+/CD38–/CD123+K12a cells and primary refractory or relapsed AML CD34+ cells, reflected by inhibition of cell proliferation and induction of apoptosis in vitro. Mechanistically, these events were associated with DNA damage accumulation and repair defects. Co-treatment with chidamide and DNA-damaging agents IDA gave rise to production of yH2A.X, inhibited ATM, BRCA1, checkpoint kinase 1 (Chk1) and 2 (Chk2) phosphorylation. Finally, the combination initiated caspase-3 and PARP cleavage and ultimately induced CD34+/CD38–/CD123+K12a cells apoptosis. 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: To observe the effect for AT101 to treat relapse and to determine its significance.

Methods: We collected data of all the APL referred to our institution from 2014 to 2017 in 21 patients. We monitored patients for 2 new diagnosis and 2 relapse of APL.

Results: Most of the patients monitored for 2 new diagnosis and 2 relapse of APL had different clinical characteristics compared to the previous diagnosis.

Summary/Conclusions: By the analysis of ROBO 1-2 and GRIP1 at the diagnosis of APL we could establish a different and strict follow-up program for patients with these alterations.

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PB1658

THE EXPRESSION OF SALL4 AND BMI-1 GENES IN MYELOID LEUKEMIA


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Background: Sal-like protein 4 (SALL4) and B-cell 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: To observe the effect for AT101 to eliminate AML stem cells and its underlying mechanism.

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+K12a 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+K12a cells and primary refractory or relapsed AML CD34+ cells, reflected by inhibition of cell proliferation and induction of apoptosis in vitro. Mechanistically, these events were associated with DNA damage accumulation and repair defects. Co-treatment with chidamide and DNA-damaging agents IDA gave rise to production of yH2A.X, inhibited ATM, BRCA1, checkpoint kinase 1 (Chk1) and 2 (Chk2) phosphorylation. Finally, the combination initiated caspase-3 and PARP cleavage and ultimately induced CD34+/CD38–/CD123+K12a cells apoptosis. 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.
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 transformed haematopoietic stem/progenitors. AML is the most common leukaemia in adults. 10-20% of AML patients lack an identifiable molecular abnormality (so-called de novo AML), while remaining patients have a wide range of genetic abnormalities that are potentially actionable.

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. We used 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 was found to vary between samples but also in comparison to OCI-AML3 cell line. In further experiments using AML patient bone marrow samples, treatment with JQ1 showed suppression of S100A8 and S100A9 in some patient samples but enhanced expression in other bone marrows. In peripheral blood samples of healthy volunteers, we found that treatment with JQ1 showed notable suppression of both S100A8 and S100A9 with a greater suppression being observed in the 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 disease. Further work may give more detailed insight into the mechanisms of action and potential use of S100A8 and S100A9 in AML prognostic markers.

SUCCESSFUL COVERAGE OF DIFFICULT TO SEQUENCE GENES (CALR, CEBPA, and FLT3) ASSOCIATED WITH MYELOID DISORDERS USING A HYBRIDISATION-BASED ENRICHMENT APPROACH PRIOR TO NEXT-GENERATION SEQUENCING

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Background: The application of short read NGS for research into myeloid disorders such as myeloproliferative neoplasms (MPNs) and acute myeloid leukaemia (AML) is limited by the inability to sequence certain challenging DNA sequences that can impact the quality of the data generated, e.g. large indels and low complexity regions (CALR), high GC content (57% on average for the whole gene with specific regions at 100%) and repetitive regions (CEBPA), and complex repetitive elements (FLT3).

Aims: To determine whether a hybridisation-based enrichment approach overcomes the difficulties associated with these genes, and permits the generation of high quality (sufficient de-duplicated depth) data to allow these targets to be accurately interrogated.

Methods: We utilised a hybridisation-based enrichment approach for library preparation in combination with a 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 ITDs of between 24 and 201 bp in FLT3.

Summary/Conclusions: This approach is suitable for the analysis by NGS of certain challenging DNA sequences that can impact the quality of the data generated, e.g. large indels and low complexity regions (CALR), high GC content (57% on average for the whole gene with specific regions at 100%) and repetitive regions (CEBPA), and complex repetitive elements (FLT3).

ASSOCIATION OF MRNA EXPRESSION PROFILES WITH FUNCTIONAL AND MOLECULAR ACUTE MYELOID LEUKAEMIA CATEGORIES

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Background: Development of high-throughput technologies such as Next Generation Sequencing (NGS) allowed the identification of recurrent mutated genes in Acute Myeloid Leukemia (AML), and new molecular markers which help refine patients’ classification in different risk groups.

Epigenetic alterations such as aberrantly expressed microRNAs (miRNAs) also play a critical role in the pathogenesis of AML. miR-NAs control processes such as cell development, differentiation, proliferation and apoptosis. Therefore, aberrant miRNA expression may affect signaling and metabolic pathways, directing cancer cell biological behavior. Recently, several studies have classified AML according to different criteria. To date, no criterion has classified AML and acute promyelocytic leukemia (APL) using a molecular approach, which could cooperate in the development of this hematologic malignancy. In conclusion, the mutational landscape of significant functional and molecular groups in AML is accompanied by miRNA deregulation, which could cooperate in the development of this hematologic malignancy.

PB1661

PROTEOMIC APPROACH TO IDENTIFY MOLECULAR TARGETS OF HALOFUGINONE IN ACUTE MYELOID LEUKEMIA

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Background: Halofuginone (HF) is a halogenated derivative of Februfin, 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 Halofuginone shows anti-leukemic properties in 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-B 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 has anti-leukemic properties in other AML subtypes of acute myeloid leukemia (AML) and HF targets were not determined yet.

Aims: Evaluate the anti-leukemic 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, MVA-11, U937 and OCI-AML3 were treated in vitro with HF at concentrations ranging from 25 to 1000 ng/ml. The % of apoptotic cells, the distribution of cells in different cell cycle phases, and the HF IC50 was determined for each cell line. We used the Proteome Profiler™ Array – HumanPhospho-Kinase Array to verify the possible tyrosine kinases and signaling pathways that could be modulated by HF. To analyze the in vivo effect of HF on cell transplantation of the leukemic cell line Kasumi-1 and THP-1, male BALB/c NOD.Cg-Pkdcricid [Ig2m1Wl/J]*SzJ (NSG) mice, which were then treated by intra-peritoneal injections of HF at a dosage of 150 mg/Kg daily for 14 days. The leukemic infiltration of the peripheral blood was quantified by flow cytometry every 2 weeks (using a anti-human CD45.2). Results: HF IC50 values ranged from 125.58 ng/ml in Kasumi-1 to 786.15 ng/ml in THP-1 cells. Kasumi-1 cells halted in the S phase of the cell cycle when treated with HF, displaying a significant decrease in proliferation, while no effect was observed for THP-1 cells. Corroborating our in-vitro observation indicating resistant of THP-1 cells towards HF, we did not detect significant difference in overall survival (OS) of NSG mice transplanted with THP-1 cells 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 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 syntase (eNOS) and Signal transducer and activator of transcription 3 (STAT3 Y705), thus suggesting that these proteins are primary targets of HF. In addition, the protein target of rapamycin (TOR) was down regulated only in THP-1, while the levels of STAT3 S727 and STAT5αβ were significantly decreased by HF treatment only in Kasumi-1 cells. This comparative analysis suggests that the sensitivity to HF may be dependent on activation 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 TET2, IDH1/2, DNMT3A, IDH2 AND CD34+ CIKES.
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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 IDH2 as well as IDH1 and CDH2, 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.

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 approx. 850 000 CpGs. AML samples were chosen based on their mutational status and divided into 4 groups: DNMT3A+ (n=3), IDH1+ (n=3), DNMT3A+/IDH1+ (n=3) and IDH2+ (n=3). The remaining DNA methylation regulatory genes as well as CEBPA were unmethylationed. 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 (IDH1, IDH2+ and CD34+ samples) and non-methylated (P<1 into DNMT3A+ IDH1+ CD34+ normal) strongly suggesting 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<0.001), while genes that are hypo- or hydroxymethylated are often CD34+ patients. In addition, we detected a subgroup of CpGs assigned to HOXA2, HOXA4, HOXA10, HOXB3, HOXC4 and HOXD3 genes that are hypermethylated in IDH1+, hypomethylated in DNMT3A+ and normally methylated in DNMT3A+IDH1+ samples relative to CD34+ normals. Clustering of DNA hydroxymethylation and methylation data resulted in the same 4 main clusters as shown for DNA methylation data.

Summary/Conclusions: We explored that AML patients with clearly defined mutational background exhibit distinct DNA methylation as well as hydroxymethylating profiles. The presence of two mutations that have the opposite effect on DNA methylation pattern (DNMT3A and IDH1) is linked to mixed DNA methylation patterns, which prevents unambiguous assignment to one cluster. Further, our data support that IDH1+ and IDH2+ represent distinct biological entities. On the contrary, gene expression profile did not support separation of samples into different mutational subgroups. We plan to enlarge the patients’ cohort and validate the most promising genes involved in selected pathways.

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Summary Conclusions: We explored that AML patients with clearly defined mutational background exhibit distinct DNA methylation as well as hydroxymethylating profiles. The presence of two mutations that have the opposite effect on DNA methylation pattern (DNMT3A and IDH1) is linked to mixed DNA methylation patterns, which prevents unambiguous assignment to one cluster. Further, our data support that IDH1+ and IDH2+ represent distinct biological entities. On the contrary, gene expression profile did not support separation of samples into different mutational subgroups. We plan to enlarge the patients’ cohort and validate the most promising genes involved in selected pathways.

PB1664 RNA-MEDIATED CORRECTION OF ABERRANT 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 demethylating agents approved for therapeutic appli- cations, 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. PNAS 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 methylation status of P15 was shown to be inversely correlated with ANRIL (Antisense Non-coding RNA in the INK4 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 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-based epigenetic interventions.

Summary/Conclusions: There is much interest in using RNA molecules as a therapeutic tool (Kole et al., Nat Rev Drug Discovery 2012; Reebye et al., Hepatology 2014). Introduction of such an approach offers greater advantages over...
existing hypomethylating-based protocols: a) high gene specificity b) lower cytotoxicity and c) absence of drug-based off-target side-effects. In the short term, this research can lead to the identification of novel key regulators of leukemogenesis and new targets for therapeutic treatments; in the long term pave the way for development of RNA-based gene demethylating agents for cancer treatment.

PB1665

**JQ1 AND CURCUMIN COMBINED TREATMENT SHOWS SYNERGIC EFFECTS IN MLL-REARRANGED LEUKEMIA CELL LINES**

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**Background:** MLL-rearranged leukemia accounts for 70% of infant and 10% of adult acute leukemias, featuring a particularly poor prognosis and high risk of relapse. Our main field of study is AML, in which nearly 50% of total cases accounts for t(9;11) translocation, the remaining 50% predominantly includes t(6;11)(q27;q23), t(10;11)(p12;q23), t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3). A 2% of AML total cases, however, is characterized by t(4;11) translocation, which is a marker of bad prognosis and it’s, so far, poorly characterized. A key feature of MLL-rearranged leukemia is cMyc overexpression, a well-known oncogene involved in several types of cancer. JQ1 is a novel molecule, which prevents cMYC expression binding an important bromodomain protein, BRD4. Moreover, Curcumin, a natural compound, inhibits p53 enzymes preventing lysine 14 acetylation on histone H3 (AcH3K14), a particular residue which is bind by BRD4 to exert its function.

**Aims:** We would like to explore a potential synergic effect between JQ1 and Curcumin molecules in the attempt to develop a novel therapeutical alternative to standard chemotherapy and to deeply investigate features underlying the molecular pathogenesis in pediatric MLL-rearranged pediatric AML.

**Methods:** Four human leukemia cell lines with MLL fusion protein have been employed in this study. RS4-11, MV4-11 expressing MLL-AF4 and THP1, MOLM13 expressing MLL-AF9 fusion genes. 5pM and 10μM Curcumin were used to treat MLL-AF4 and MLL-AF9 cell lines respectively, while 250nM JQ1 was used to treat all the cell lines. After 2 days of treatment, either with single and combined drugs, cell number quantification, based on metabolic activity, was detected through XTT assay. In order to assess the cMYC, CDKN1A, BCL2 transcripts levels and mir-99a expression a quantitative RT-PCR analysis was carried out, while we used western blotting to detect the expression of cMYC, PARP, Caspase3 and AcH3K14. Apoptosis and cell cycle were evaluated by flow cytometric analysis.

![Figure 1](image)

**Results:** In apoptosis analysis, a synergic effect was detected for all 4 cell lines, similarly cell cycle evaluation showed a significant accumulation of cells at SubG1 phase (2-8 fold) (Figure 1). XTT metabolic assay showed a reduction in proliferation percentage: 65±5 for curcumin and JQ1 single treatment and 59±5 for combination of drugs in both MLL-AF4 cell lines, meanwhile in MOLM13 cells it was 64±2 and 87±2 for curcumin and JQ1, respectively and 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 t(4;11) translocated cells, considering the more intense effect of the combined drugs on previous analysis. qRT-PCR and western blot experiments revealed a synergic effect of the 2 experimental drugs on both apoptosis and proliferation gene related (bcl2, caspase3, Parp, cdkn1a) as well as on 4 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 TP33F EXPRESSION LEVELS IN RELATION TO NPM1 AND CEBPA MUTATIONS**

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**Background:** Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with the presence of diverse genetic abnormalities in hematopoietic stem cells. The most frequent alterations in normal karyotype AML (NK AML) are mutations in exon 12 of nucleophosmin gene (NPM1). Until now 56 different mutations of NPM1 exon 12 have been described, mostly insertions. The NPM protein plays an important role in cell cycle and apoptosis control. It cooperates with several proteins, among them with p53 and ARF. The median levels of functional nuclear p53 protein are reduced in NPM1 and FLT3 ITD mutant samples. TP53 encodes a tumor suppressor protein which consists of transactivation, DNA-binding and oligomerization domains. Due to alternative splicing it may exist in 13 different isoforms. Alternative splicing of intron 9 leads to production of 2 different proteins, p53β and p53γ, without oligomerization domain (stop codon is localized in exon 9b). These isoforms can be present in acute myeloid leukemia (AML) cells. p53β binds to BAX promoter and can induce apoptosis independent from p53 wt. p53 has influence on activation of CEBPA which is associated with cell cycle regulation, especially cell cycle arrest and plays also role in cell differentiation. Generally, it is a transcription factor expressed during myeloid lineage development, from progenitor cells to mature granulocytes. Various mutations of CEBPA gene are described. Among them N-terminal and C-terminal mutations, mostly insertions and deletions, are often present.

**Aims:** The goal of the study was to assess mutual status of NPM1, CEBPA and FLT3 in association with TP53beta and TP53gamma expression levels.

**Methods:** 75 NK AML patients were included in the study. NPM1, CEBPA and FLT3 gene mutations were analyzed by direct sequencing. TP53β and TP53γ expression levels were assessed real time PCR. Expression levels were analyzed with ΔΔCt method, with ABL as a control gene and K562 cell line as a calibrator.

**Results:** In all 75 cases, TP53β and TP53γ transcripts were detected. 36 patients had NPM1 mutations, 25 had CEBPA mutations or known polymorphisms, and 25 had FLT3 ITD mutation. Assessed median expression level of TP53β was much higher (ΔΔCt 43,11) than TP53γ (ΔΔCt 10,85; p<0,05). Furthermore, expression level of TP53γ in CEBPA mutated group (ΔΔCt 11,4) was significantly lower than in CEBPA wt group (ΔΔCt 17,7) (p=0,03). We have not found any other important correlation between mutations of studied genes and TP53 expression. We also classified patients according to median expression value of TP53, to two groups: with overexpression or with low expression. Haematological and clinical features, such as white blood cells count (WBC), blasts count in bone marrow or patient age did not depend on TP53 isoform expressions. However, statistical analysis showed important difference between WBC count in NPM1mutated and NPM1wt groups.

**Summary/Conclusions:** Obtained results may suggest a clinical importance of simultaneous analysis of TP53 isoform expression and mutations in CEBPA gene. It may be hypothesized that a changed sequence of the latter gene might influence TP53 isoform expression and in consequence regulate the cell cycle.

PB1667

**EXPRESSION PROFILE OF EPIGENETIC MODULATORS IN ACUTE MYELOID LEUKEMIA OF INTERMEDIATE RISK**

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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 (Beti)-results in leukemic 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 be more complex. It has been recently suggested that leukemic effect of Beti could be due to c-myc suppression and also that high Bcl-2 levels may target those patients that would benefit from Beti. We believe that establishing the expression profile of epigenetic modulators in AML may help in the identification of patients that could benefit from Beti.

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 Bcl2 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 as a control gene.

Results: Levels of BRD4 mRNA were positively associated with EZH2 (Spearman’s = 0.285, p=0.021) and BRD4 with c-myc (Spearman’s coefficient=0.420, p<0.001). Bcl2 (Spearman’s =0.471, p<0.001) EZH2 (Spearman’s =0.4655, p=0.008) and ASXL1 (Spearman’s=0.9498, p<0.001). Survival analysis considering 50th percentile as a cut-off value for BRD4 expression indicated that patients with higher levels of BRD4 had better overall survival (median OS of 27 months, 95% IC 15-38.9) 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 BRD4 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 inverse association of BRD4 expression level with c-myc mRNA in 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 are necessary to confirm the relationship between higher BRD4 levels and better overall survival. Finally, future analysis should be done to determine whether patients with higher BRD4 expression levels determine a subgroup with better response to Beti.

PB1686

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 CEBPA+ AML.

Methods: Aims: Thirty-two patients with de novo AML and CEBPA+ 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, glycoporin, CD71, CD11b, myeloperoxidase, CD79a, TdT, lysosome 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 InfiFlow 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, MLPTD, 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%), CD13/37/39,95%, CD56 (8/39,15%), 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 GATA2 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: CEBPA+dm cases showed an homogeneous immunophenotype with positivity for CD45, CD7, CD34, CD123, CD13, HLA-DR, CD71, CD33, CD13 and CD15. CD36 and/or CD56 overexpression was detected in a subgroup of cases (9/39, 23%) with an adverse outcome. The current findings suggest that CD36 and CD56 reactivity should be investigated in larger series of CEBPA+ AML cases. Small leukemic populations with B-cell markers are not uncommon in CEBPA+ 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 346 spots in PB samples after induction therapy, reflecting changes in protein expression due to treatment. Comparing the proteomes, we found 11 spots that differed significantly (fold change of +/- 1.5 and p < 0.05). Of these, seven proteins were up-regulated and four were down-regulated at time of diagnosis (before treatment) compared to after induction treatment. Nine of these spots corresponded to low molecular weight proteins (<40 kDa) and 2 spots have a molecular weight between 40-60 kDa. Based on the molecular weight and isoelectric point information of these spots we were able to search for proteins reportedly involved in leukemia, in order to propose possible identities (see Table 1). In terms of biological process, 4 proteins (α-Enolase, HSP27, 14-3-3 protein zeta/delta, and GST-P) are involved in the regulation of apoptosis. The F-actin-capping protein subunit beta-2 like 2 is a positive regulator of histone acetylation and DNA repair. GRPB2 is a positive regulator of histone acetylation and DNA repair. GRPB2 is a known up-regulated target of AMPK. Our results indicate that the AMPK activator might be a potential therapeutic target for leukemia.}

**Table 1.**

<table>
<thead>
<tr>
<th>Spot</th>
<th>Fold Change</th>
<th>%/Relative Mass</th>
<th>%/Relative MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC1</td>
<td>2.5</td>
<td>78%</td>
<td>30 kDa</td>
</tr>
<tr>
<td>HC2</td>
<td>1.8</td>
<td>65%</td>
<td>40 kDa</td>
</tr>
<tr>
<td>HC3</td>
<td>1.6</td>
<td>50%</td>
<td>50 kDa</td>
</tr>
</tbody>
</table>

**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 CHROMEOSSISTATE AML CELLS**

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**Background:** Cytarabine is a chemotherapeutic drug used alone or in combination with other anticancer drugs to treat acute myeloid leukemia (AML). New treatment strategies are emerging to enhance the anti-cancer effect and decrease the toxicities. Nucleophosmin (NPM1 or B23) is a ribosomal protein localized in the nucleolus and multifunctional enzyme in cancer metabolism and protein synthesis. AMP-activated protein kinase (AMPK) is a critical energy sensor to regulate homeostasis and plays a potential role for anti-cancer proliferation and apoptosis in acute myeloid leukemia(AML) THP-1 cells.

**Methods:** 1) We cultured human AML THP-1 cells. 2) The cells were incubated with different concentration of cytarabine for 24/48 h, and the cell viability was measured by cell counting kit-8(CCK-8) to determine the IC50 of cytarabine. 3) The cell cycle distribution was measured by Annexin V-FITC/PI double staining flow cytometry.(FCM)

**Results:** The protein expression levels of NPM1, TRF1, TRF2 were measured by immunohistochemistry(IHC) staining. The protein expression levels of POT1 and TRF2 were measured by western-blotting.

**Summary/Conclusions:** Our results suggest that the higher concentration of cytarabine upregulates NPM1 overexpression level in AML cells. 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 of 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) We cultured human AML THP-1 cells. 2) The cells were incubated with different concentration of quercetin for 24/48 h, and the cell viability was measured by cell counting kit-8(CCK-8) to determine the IC50 of quercetin. 3) The cell cycle distribution was measured by Annexin V-FITC/PI double staining flow cytometry.(FCM)

**Results:** We found that quercetin significantly suppressed THP-1 cells proliferation in dose- and time-dependent manner. Treatment with quercetin significantly increased THP-1 cell line apoptosis and G1 phase arrest rate. Furthermore, the protein expression levels of POT1, TRF1, TRF2 were decreased. The mRNA expression levels of POT1, TRF1, TRF2 were consistent with their protein expression levels, respectively.

**Summary/Conclusions:** We firstly demonstrated 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.
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 inhibit 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.

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**Acute myeloid leukemia - Clinical**

**PB1673**

IN VITRO DRUG SENSITIVITY TEST IN THE INDIVIDUALIZED ANTI-LEUKEMIA CHEMOTHERAPY FOR THE NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA


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**Background**: The biological properties, genetic abnormalities of leukemic cells influence on their sensitivity to chemotherapeutic drugs. It is widely known that there can be significant differences both in genetic features as well as in drug resistance profile of individual tumors with the same phenotype.

**Aims**: The purpose of this study was to analyze the relationship between in vitro chemosensitivity test results using the Cell Titer-Glo assay and clinical response on chemotheraphy, and to find the possibility of optimizing the treatment for individual patients according to their actual drug resistance.

**Methods**: For The Cell Titer-Glo assay, we obtained bone marrow aspirates or peripheral blood samples from 66 patients with newly diagnosed acute myeloid leukemia at the time of initial diagnosis. The following drugs were tested: cytarabine arabinoside, daunorubicin, idarubicin, fludarabine, etoposide, and methotrexate. We evaluated clinical response and survival outcome according to chemosensitivity of drugs and protein expression.

**Results**: In this study, in vitro chemosensitivity test with the Cell Titer-Glo assay showed the relationship between chemosensitivity and survival outcome significantly. The 5-year overall survival rates with dichotomized chemosensitivity of idarubicin (64.6% vs 33.3%, p=0.046), cytarabine (63.7% vs 63.3%, p=0.0291), and fludarabine (80.1% vs 37.5%, p=0.020) were higher in low concentration level than in high concentration level. There was a tendency of higher relapse-free survival rate at 4-year in the patients with low level IC50 than in the high level IC50. However, cytotoxic effect of testing drugs in vitro by the Cell Titer-Glo assay did not show a relationship with complete remission rate after induction and leukemia recurrence rate.

**Summary/Conclusions**: Although the Cell Titer-Glo assay did not provide the prediction of clinical response of induction treatment, it can be a useful tool in individually optimizing the chemotherapy of patients with newly diagnosed acute myeloid leukemia.

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**PB1674**

PROGNOSTIC IMPACT OF P53 EXPRESSION IN BONE MARROW BIOPSY OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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**Background**: Several studies have shown that the presence of the TP53 mutation is related to an unfavorable prognosis in patients with acute myeloid leukemia (AML). However there are few reports on the evaluation of its expression by immunohistochemistry in bone marrow (BM) biopsy.

**Aims**: To evaluate the expression of p53 in BM biopsy of AML patients at diagnosis and its impact on survival.

**Methods**: This retrospective analysis included 85 patients with de novo AML diagnosed from January 2005 to December 2015 submitted to BM biopsy at diagnosis. p53 expression was detected by immunohistochemistry, and staining was evaluated using the H-score (range 0–300). The t-test and Mann-Whitney U test were used to detect differences in the distribution of continuous parametric and nonparametric variables, respectively. Overall survival (OS), disease-free survival (DFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method. The log-rank test was used for comparison of survival curves. The interaction between the examined prognostic variables was tested with univariate and multivariate Cox regression analysis.

**Results**: Median age was 60 years (17–81). There was a predominance of patients >60 years (54.1%) and males (56.5%). The median H-score for p53 was 11.8 (0.4–161.1), with no significant correlation with age or cytogenetic risk. p53 expression was significantly higher in patients with a complex karyotype (p=0.0031) and high risk by European Leukemia Net (ELN) criteria (p=0.047).

There was a positive correlation with complex karyotype and prognostic risk by ELN. Excluding early deaths (<30 days from induction), patients younger than 60 years with H-score >60 showed worse overall survival when compared with patients with H-score <60 (0% vs 14.6%, respectively) (p=0.048). There was no statistical difference in disease-free survival and event-free survival. In the Cox univariate analysis including all cases, peripheral leukocyte counts at diagnosis (p=0.014), cytogenetic risk groups (p=0.07), ELN risk categories (p=0.023) and H-score (p=0.025) were significant. In a multivariate model including leukocytosis, ELN risk and p53, all variables remained in the model.
Summary/Conclusions: Expression of p53 assessed by immunohistochem- istry is a fast, objective and promptly available tool for prognostic evaluation of AML. A high expression of p53 (H-score >60) was related to a lower overall survival in de novo AML.

PB1675

Abstract withdrawn.

PB1676

LONG-TERM FOLLOW-UP OF SALVAGE TREATMENT FOR RELAPSED AML WITH CLADRIBINE, HIGH DOSE CYTARABINE AND IDARUBICIN

Background: Despite improving response rates in induction treatment for AML during the last years the outcome for relapsed or refractory AML is still poor. Currently, no standard therapy exists for patients with relapsed AML. Furthermore, CR rates are lower than in newly diagnosed patients and range between 15% and 50%. There is evidence that the ELN-risk-group 2017 3/14 (22%) had favourable cytogenetic changes, 9/14 (64%) intermediate and 2/14 (14%) belonged to the adverse cytogenetic group. The performance status was good in most patients (ECOG 0 in 10%, ECOG 1 in 80%), but reduced (ECOG 2) in 20% (5%) patients. After the first course, CR/CRi was achieved in 60% and PR in 10% of patients. Median duration of neutropenia was 18.5 days (range 14-24d). The main grade 3 or 4 non-haematologick toxicity was infection seen in 85% of cases. Nausea occurred in 30%, hepatotoxicity, mucositis and diarrhea in 11% of cases. Cardiac or renal tox- icities grade 3/4 were not observed. Two patients (10%) died due to infection. Six patients received a second course of CAI/CA. Altogether, 6 patients were refractory. Nine patients (48%) proceeded to allogeneic stem cell transplanta- tion after induction therapy with CAI. Of those, 4 patients are still alive and free of leukemia and one patient died CR in 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 EVI1 EXPRESSION PREDICTS POOR OUTCOMES IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS WITH INTERMEDIATE CYTOGENETIC RISK RECEIVING CHEMOTHERAPY ONLY

Background: Nearly half of acute myeloid leukemia (AML) patients are defined as an intermediate cytogenetic risk, however the patients in this group have greatly varied outcomes and need to be stratified. Apart from gene mutation, abnormal gene expression might also be prognostic, and ecotropic viral integra- tion site 1 (EV11) expression is a representative. To date, the poor prognostic impact of EVI1 expression in AML has been reported, but almost all studies have been undertaken by European researchers. EVI1 prognostic significance in AML remains to be confirmed in other populations. Furthermore, because the selection protocol and cutoff values selection methodologies differed among studies, the threshold for defining EVI1 high expression remains obscure, which hinders its clinical routine application.

Aims: We investigated the prognostic impact of EVI1 transcript levels in Chi- nese adult intermediate cytogenetic risk AML (ICR-AML) patients who received chemotherapy only in a single center. The appropriate cutoff values for grouping EVI1 expression were also evaluated.

Methods: A total of 191 adult patients receiving chemotherapy only were includ- ed in this study. They were diagnosed as ICR-AML according to morphology, immunophenotyping, cytogenetics and molecular biology. Their bone marrow samples were collected at diagnosis. Real-time quantitative PCR was per- formed to test EVI1. MLL partial tandem duplicate (MLL-PTD) and WT1 tran- scripts, and their transcript levels were calculated as the percentage of target transcript copies/ABL copies. NPM1 mutations and FLT3 internal tandem dupli- cation (FLT3-ITD) were individually screened by real-time quantitative PCR and qualitative PCR. 27 normal bone marrow (NBM) samples form volunteers were simultaneously tested EVI1, MLL-PTD and WT1 transcripts. All partici- pants provided written informed consent in accordance with the Declaration of Helsinki.

Results: The upper limit of EVI1 transcript levels in 27 NBM samples was 8.0%. Receiver operating characteristic curve analysis showed that 1.0% (a 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- ment. EVI1<1.0% was significantly associated with longer 2-year relapse-free survival (RFS), disease-free survival (DFS) and overall survival (OS) rates in the entire cohort (P=0.0003, 0.0017 and 0.0009), patients with normal karyotypes (n=148, P=0.0032, 0.0047 and 0.0007) and FLT3-ITD (-) patients (n=150, all P<0.0001). Multivariate analysis showed that EVI1≥1.0% and FLT3-ITD (+) were independent adverse prognostic factors for RFS (Table 1), DFS and OS in the entire cohort. In addition, patients with EVI1 between 1.0% and 8.0% had 2-year RFS rates similar to those with EVI1>8.0% (P=0.16), and both patient groups had significantly higher RFS rates than those with EVI1<1.0%.

Table 1.

<table>
<thead>
<tr>
<th>EVI1 expression</th>
<th>HR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1.0%</td>
<td>4.0 (2.1-7.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FLT3-ITD (+)</td>
<td>3.4 (1.9-6.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FLT count</td>
<td>&lt;100/1000FL/L</td>
<td>2.1 (1.1-4.3)</td>
</tr>
<tr>
<td>Blast percentage in BM &gt;65%</td>
<td>2.1 (1.3-3.6)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Summary/Conclusions: EVI1 transcript levels at diagnosis could further strat- ify adult ICR-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 National Science Foundation of China (81370657, 81370639 and 81570130).

PB1678

EFFICACY AND SAFETY OF DECITABINE IN ELDERLY AML PATIENTS: A REAL LIFE MULTICENTER EXPERIENCE OF THE NETWORK RETE EMATOLOGICA LOMBARDIA

Background: The optimal treatment decision in older patients (pts) with AML remains controversial, especially in patients pts with comorbidities, non-fit to intensify therapy or with AML adverse biologic features. Recently decitabine was approved in Italy in AML pts unfit to chemotherapy aged >65 years (y) and could be adopted in a population based setting.

Aims: To evaluate efficacy and toxicity of decitabine in a consecutive series of elderly AML pts (no M3), considered unfit to chemotherapy (CT) according to Ferrara et al (Leukemia, 2013) and treated at 6 centers of the Hematological Lombardy Network (REL).

Methods: Between Dec 2015 and Dec 2016, 46 (F/M: 22/24) newly diagnosed AML pts were included. 25 pts (54%) had a previous history of chemotherapy for >65 years (y) and 14 pts (30%) had no previous chemotherapy. 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 intensive CT, 1 frail and 4 fit. Unfitness causes were age >75y (58.5%), PS ECOG≥3 in 10.8%. According to “fitness”, 41 pts (89.1%) were defined unfit to chemotherapy (CT) according to Ferrara et al (Leukemia, 2013) and treated at 6 centers of the Hematological Lombardy Network (REL).

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pts., according to ELN (Doehner, 2017). In 2 pts it was not evaluable. Molecular analysis was available in 17/19 NK; NPM1 was mutated in 5 cases, with (2) or without (3) FLT3-ITD mutation.

Results: The total number of cycles administered was 231 (median 3.5; range 1-20). In 37/46 evaluable pts (2 ongoing, 1 early and 8 aplastic deaths), overall response rate (ORR) and complete remission (CR) rate were 51% and 32%, respectively. Partial response (PR) and hematological improvement were achieved in 5.5% and in 13.6%, stable disease in 29.9% and failure in 19% of pts, respectively. Median time to best response was 3.5 months (range 1-8.5). Median response duration was 5.3 months (1-18+ ms) and median overall survival was 8.4 months and projected OS at 1 year was 54%.

Re-induction CLAM comprised clofarabine (40mg/m2/day, days 1-5), cytarabine (12mg/m2/day, days 3-5) and mitoxantrone (750mg/m2/day, days 1-5) and mitoxantrone (12mg/m2/day, days 3-5). 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). Survivals were determined using Kaplan Meier analysis.

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receiving 105 cycles of chemotherapy between June 2012 and December 2016 in Masaryk University Perník Research and Training Hospital. Median patient age was 50 years (18-73); and there was no significant gender difference (38 female vs 44 male (46% vs 54%)). All patients had active disease, 78 (74.3%) of them received 3+7 (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 8.9 (9-34) days if IFI under 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 (17.1%). Discontinuation reasons were due to adverse events in 6 cycles (5.7%), and due to other reasons (diarrhea, intolerance of oral medication, recurrent high grade fever, death) in 39 cycles (37.1%). IFI incidence under effective posaconazole prophylaxis was 28.1% (18/64). Total clinical failure rate was 60% (63/105). IFI was diagnosed with pulmonary nodules in 12 of 18 patients (66.6%), EORTC-MSG: possible, 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, the overall case-mortality rate at day 100 was (8/44; 20.4%) significantly lower than patients unable to continue posaconazole prophylaxis (12/26; 46.1%) (p=0.023). In the subset of patients receiving prophylaxis as planned; there was no statistically significant difference in IFI incidence between previously untreated AML (13/46; 28.2%) and relapsed/refractory AML (5/18; 27.7%).

Summary/Conclusions: In our real-life experience, we have demonstrated early effective and well-tolerated 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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1Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russia

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 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 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 (53 male / 116 female). The median age at diagnosis was 52 years (22-88 years). To determine FLT3 gene mutations we used the method of polymerase chain reaction (PCR) with subsequent restric-

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 anthra-
ycine antibiotics (AA), is one of the biggest problems in treatment of patients with acute leukemia (AL). Chemotherapy with AA is accompanied by systemic endothelial dysfunction, increasing the cardiovascular toxicity risk and promoting vascular complications. Patients with co-morbid ischemic heart disease (IH) are at extremely high risk of myocardial injury and in need of anthracycline cardiotoxicity (AC) prevention.

Aims: To assess the effectiveness of L-arginine in the prevention of endothelial dysfunction as a predictor of acute AC in patients with AL and co-morbid ischemic heart disease.

Methods: A total of 66 patients with newly diagnosed acute leukemia (acute lymphoid leukemia – 7 patients, acute myeloid leukemia – 59 patients) and co-
morbid ischemic heart disease were included in the study. The cohort consisted of 46 (69.7%) males and 20 (30.3%) females. The median age was 60.7 (10-72) years, ECOG I-II. The duration of IH ranged from 3 to 15 years. Chemotherapy (CT) schemes included AA (doxorubicin). The evaluation of endothelial dysfunction was performed by determining the stable metabolites of nitric oxide – nitrite anions (NO3−) and activity of total NO-synthase in serum of patients before the CT and year after (72 weeks). To induce acute cardiac damage we used a cumulative dose of AA from 100 to 200 mg/m² by doxorubicin. The mean total cumulative dose of AA reached 162,04±24,65 mg/m² and 166,49±27,34 mg/m² in groups I and II respectively. The study was approved by the local ethical committee and all patients gave a written consent before they were included in the study. Patients were divided into two groups: (n=36) AL patients treated with CT; II (n=30) – AL patients, whom during the CT in order for prevention of acute AC were given L-arginine hydrochloride 4.2% 100 ml IV the day before and during administration of AA, followed by oral L-arginine asparatate for a month.
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 vasodilatation mechanism and endothelial dysfunction intensification. Provided achieving low cumulative dose of AA after 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 impairment, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilatation, thus reducing the risk of early anthracycline cardiotoxicity development.

PB1684

CLINICAL CHARACTERISTICS AND SURVIVAL OUTCOMES IN ACUTE ERYTHROID LEUKEMIA (AML-M6): AML/MDS WORKING PARTY STUDY OF KOREAN SOCIETY OF HEMATOLOGY


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Background: Acute erythroid leukemia is a morphologically distinct and rare entity designated as M6 in FAB classification. In Korea, patients with AML-M6 have been treated as acute myeloid leukemia with intensive chemotherapy, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilatation, thus reducing the risk of early anthracycline cardiotoxicity development.

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 45 – 54, 34 patients (40.5%); age 55 – 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 results were available in 80 patients. Among them, karyotype was normal in 43 (53.8%) and complex in 13 (15.5%) patients. 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, 27 months, 7 months, respectively, P=0.026) (Figure 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 impairment, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilatation, thus reducing the risk of early anthracycline cardiotoxicity development.

PB1685

PREGNANCY ACCUMULATES UNFAVORABLE MOLECULAR GENETIC AML AND SHOULD BE CONSIDERED AS A POOR PROGNOSTIC FACTOR

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Background: Acute myeloid leukemia (AML) during pregnancy – is a rare clin-
Clofarabine is a nucleoside analogue of 2-phenoxyethyl-2'-deoxyapurine-2'-β-D-arabinofuranoside. It is a novel nucleoside analogue of 2′-deoxyapurine nucleosides. It has been in clinical trials for the treatment of acute myelogenous leukemia (AML) and other hematologic malignancies. Clofarabine has been found to have activity in patients with relapsed/refractory AML. It has been shown to have activity in patients with relapsed/refractory AML, particularly in those who have failed previous cytotoxic chemotherapy. Clofarabine has also been shown to have activity in patients with acute lymphoblastic leukemia (ALL), and in patients with chronic myeloid leukemia (CML) in blast crisis.

**Clofarabine in Relapsed/Refractory Acute Myelogenous Leukemia: A Single Centre Experience**

**Aims:**
- To assess the safety and efficacy of clofarabine in patients with relapsed/refractory AML.
- To evaluate the impact of clofarabine on the management of patients with relapsed/refractory AML.

**Methods:**
- The study was conducted at a single centre, where clofarabine was administered to patients with relapsed/refractory AML.
- The patients received clofarabine at a dose of 22.5 mg/m² daily for 5 days.
- The response to clofarabine was assessed using standard criteria.

**Results:**
- Of the 20 patients enrolled, 16 achieved a complete remission (CR) and 4 achieved a partial remission (PR).
- The median overall survival of the cohort was 115 days.
- Among the responding patients, 16 (24%) underwent allogeneic bone marrow transplantation; in these selected patients, median overall survival was 185 days.

**Summary/Conclusions:**
- Clofarabine appears to be an effective and well-tolerated agent in patients with relapsed/refractory AML.
- Further studies are needed to determine the optimal dose and schedule of clofarabine for the treatment of AML.

**References:**
- Florence, Italy

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**Patients and Methods:**
- The study included 20 patients with relapsed/refractory AML who received clofarabine at a dose of 22.5 mg/m² daily for 5 days.
- The patients received clofarabine in combination with high-dose cytarabine.

**Results:**
- Of the 20 patients, 16 achieved a complete remission (CR) and 4 achieved a partial remission (PR).
- The median overall survival of the cohort was 115 days.
- Among the responding patients, 16 (24%) underwent allogeneic bone marrow transplantation; in these selected patients, median overall survival was 185 days.

**Summary/Conclusions:**
- Clofarabine appears to be an effective and well-tolerated agent in patients with relapsed/refractory AML.
- Further studies are needed to determine the optimal dose and schedule of clofarabine for the treatment of AML.

**References:**
Background: IRAIN which is produced from the insulin-like growth factor type I receptor (IGF1R) imprinted locus is a newly identified IncRNA. There are very little knowledge about the specific role of this IncRNA in tumorigenesis 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 was 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 groups (p≤0.05).

Summary/Conclusions: Our results suggest that down-regulation of IRAIN IncRNA might play a role in the AML development and hence may be a potential prognostic factor and serve as therapeutic target for AML treatment.

PB1689

PERFORMANCE OF THE LEUKOSTRAT® CDX FLT3 MUTATION SIGNAL RATIO ASSAY TO DETECT INTERNAL TANDEM DUPLICATION AND TYROSINE KINASE DOMAIN MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) in general has a poor prognosis. Assessment of the mutation status of the FLT3 (fms related tyrosine kinase 3) receptor gene in AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of FLT3 activating mutations portends a poor prognosis. The LeukoSTRat® CDX FLT3 Mutation Assay targets regions of the FLT3 gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations. Since this assay is a signal ratio (SR) assay with a validated cutoff of 0.05, demonstration of international harmonization of results is paramount. FLT3 ITD mutations are caused by duplication and insertion of a portion of the FLT3 gene that includes the region in and around the juxtamembrane region of the FLT3 gene. These mutations vary in both the location and the length of the inserted duplicated DNA sequence. ITD mutations result in constitutive autophosphorylation and activation of FLT3. FLT3 TKD mutations are induced by nucleic acid substitutions and/or deletions that result in a change in the amino acid sequence in this highly-conserved catalytic center. TKD mutations, such as D835 and I836 substitutions and deletions, result in constitutive autophosphorylation and activation of FLT3.

Aims: To assess the performance of the Invivoscribe® LeukoSTRat® CDX FLT3 Mutation Assay.

Methods: White blood cells were removed from peripheral blood after 30 minutes of centrifugation at 2000 xg 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 amplicons were analyzed via capillary electrophoresis. The assay measured the ratio of signals from mutation against a background of a wild type, which is produced from the insulin-like growth factor type I gene. 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 in the ITD assay detected allelic ratios of 0.03, 0.05, and 0.53 above the LoB SR in more than 95% of samples for insertions sized at 30 bp, 126 bp and 279 bp, respectively. The limit of detection in the TKD assay detected an allelic ratio of 0.05 above the LoB. For precision and reproducibility, the SR%CV was within 3-14% across ITD and TKD mutation types regardless of reagent lots, equipment or operator. There was 100% agreement between all three clinical LabPMM laboratory sites.

Summary/Conclusions: This robust assay produced a SR%CV less than 15% regardless of reagent lot, equipment or operator. The high reproducibility between the three laboratories on three different continents provides evidence that the Invivoscribe® LeukoSTRat® CDX FLT3 Mutation Assay is an internationally standardized assay.

PB1690

CLINICAL FEATURES AND OUTCOME OF PATIENTS WITH CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid leukemia is classified into different prognostic groups according to their cytogenetic profil; AML with t (8; 21) or inv(16) (16; 16) called AML CBF belong to a prognostic group of low-risk; they represent 15% to 20% of the AML.

Aims: The aim of this study is to present clinical , cytogenetic features and outcome of this group of patients (pts) in an emerging country.

Methods: Cytologic diagnosis of AML CBF is completed by immunophenotypic and cytogenetic analysis:
t(8; 21), inversion 16(t(16;16)) and del(16q22. Induction treatment: Daunorubicin 45 to 90 mg/m² day (d1-d3)+Cytarabine 100 mg/m² (d1-d7) (with progressive doses if major leukocytosis). Assessment between d 21 and d 28 by bone marrow analysis; If failure a study. The remaining pts are subjected to induction chemotherapy with a high dose of Cytarabine (PB1690)

Results: From 2010 to 2016, cytogenetic analysis was performed in all cases of AML which 58 cases(18,6%) of LAM - CBF were diagnosed. The male to female ratio was 0.5:1. A median survival time of 37 years (16-72); t(8;21) was found in 28 pts (16 M,12F); inversion (16)(p13.11;q22.21), t(16,16)(p13.11;q22.21) and del 16q22 were found in 30 pts (12M,18F), respectively, in 27 pts, 2 pts and 1 pt. Four cases of del(16)(p13) were associated with inv(16). For inv(16), FAB subtypes were AML4 (26). AML (2) and AML; For t(8;21), there was 26 AML2 and 2 AM3. Evaluation of induction: not evaluable: 13 cases, Complete Remission (CR): 38 cases (65,5%); for 7 cases in failure , a second induction was proposed, we obtained 2 CR. 15 pts were transplanted. Outcome: 27 pts are alive in CR of which 12 transplanted . 31 pts died of which 18 toxic deaths ( 15 after induction treatment and 3 after engraftment). Median overall survival for inv(16) : 11 months vs 15 months for t(8;21) (p=0,87).

Summary/Conclusions: In our study, the frequency of the CBF AML is closer than those described in another Algerian study and literature: 18,6% vs 15.4% and 20% respectively; a slight predominance of the inv 16 or t (8;16) identical translocation. This mutation FLT3, advanced age, the leukocytosis, severe thrombocytopenia and failure a study. The remaining pts are subjected to induction chemotherapy with a high dose of Cytarabine (PB1690)

PB1691

FLOW CYTOMETRY ANALYSIS SOFTWARE FOR REMOTELY LOCATED HAEMATOLOGISTS

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Background: Current flow cytometry software packages are unsuitable in cases where the interpreter of the data isn’t physically located at the computer with the software installed. This is particularly disadvantageous in urgent situations, such as in the diagnosis of acute leukemia.

Aims: Develop a tool to allow haematologists to analyse flow cytometry data from anywhere on any internet-enabled device e.g. tablet, smartphone, laptop, PC.

Methods: We came up with principles a new software package should adhere to: 1. should be accessible from any Internet-enabled device e.g. iPad, Android phone, Blackberry, laptop; 2. should not require installation; 3. FCS data should be anonymised; 4. data transfer should be secure and encrypted; 5. software must include all basic functionality of flow cytometry software e.g. dot plot graphs, histogram graphs and gating 6. should put collaboration to the forefront e.g. analysis can be instantly linked to via a web URL.

Results: The resulting software package is a web app which is accessible from any internet-enabled device e.g. smartphone, tablet, laptop or PC. On mobile devices such as an iPad, touch is used for drawing of gates, selection of quadrants, selections of parameters etc. On laptop’s and PCs, these are drawn via
the mouse or keypad. The software utilizes 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—lab technician running the sample can upload the sample and instantly shared 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 to 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, Idarubicin 12 mg/m2 days 1-3, ara-C 2 mg/m2 days 1-5) in these patients.

Aims: To evaluate our response rates and the survival with FLAG-IDA protocol.

Methods: Descriptive study of a case series of patients with acute leukemia who received intensive induction chemotherapy with FLAG-IDA protocol at our hospital between January 2007 and December 2016. Biodemographic, histopathological, cytogenetic and molecular results and previous treatment were recorded. We analyzed the response rate, the 30-day mortality rate and the overall survival.

Results: 65 patients received treatment with FLAG-IDA protocol between 2007-2016, 36 of them female, with an 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 (disease relapse ≥23 days). Based on European Prognostic Score (EPI-SCORE) for patients with RR-AML, 61% of them had a poor prognosis (10-14 points), 36% had an intermediate prognosis (7-9 points) and only 3% had a favorable prognosis (1-6 points). The next important group, 25% (n=17) were MDS patients transformed to AML. We had 9% (n=6) patients with treatment related AML and 6% with other acute leukemia (3 cases of refractory ALL and 1 case of biphenotypic leukemia). We observed a global response rate of 63%; 51% (n=33) of patients had a complete response (CR) and 12% (n=8) partial response, 17% (n=11) did not have a response and 20% of patients were not evaluated after to receive the treatment because they had a early death. The 30-days mortality rate was 21.5% (n=14), similar to the 30% observed 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).

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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 the CNS, 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 5.10⁹/L; hemoglobin 99 g/L; platelets 116.10⁹/L. CD56+, CD123+. No standard therapies were applied. Patients received CHOP and 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: 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 regimens 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 months, Max: 13.8 years). 12 patients were excluded from further analysis for not being able to complete induction phase. Donor’s syndrome, 7.7% (11 of 143) had concomitant malignancies. 85.7% (120) of CNS-1, 27.4% (20 of 73) had MLL Gene rearrangement, 21.2% (14 of 66) were positive for TELAML/RUNX1/RUNX1T1 and 22% (13 of 59) had PML/RAR (+). Trisomy 14, 10 or 17 was not seen among any of 13 patients tested. Most commonly observed FAB classification was M-5 (23.9%, 24 of 102) followed by M-2 (18.6%). 27.3% (39) were Low Risk, 43.4% (62) Intermediate and 29.4% (42) High Risk. 43.3% (58 of 134) received HSCT.

Results: Our CR-1 rate was 93.7% (143 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 (0.56±0.046); significantly poor (P-Value: 0.001) in relapsed (n=52, 0.197±0.051) compared to non-relapsed (n=82, 0.86±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 (0.16±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, 0.82±0.070) compared to relapsed patients (n=27, 0.16±0.073; P-Value: 0.003) for whom HSCT 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 also described to be responsible for solid tumors chemoresistance, invasion, metastasis and relapse. The role of HIFs in leukemias has not been established yet. First reports of poor outcomes of antileukemic treatment linked with overexpression of HIF-1a has been published. Moreover another HIF subunit - HIF-2 alpha - has been described in mouse model as increasing myeloid preleukemic cell proliferation and accelerating disease progression with reduced survival. On this background, we found interesting if HIFs-alpha expression in cancerous leukemia correlates with progression in human.

We have tried to find a connection between AML cells percentage expressing HIF-2 alpha and first line chemotherapy results.

Aims: The aim of the study was to determine the role of HIF-2 alpha in human AML.

Methods: We analyzed a 26 primary AML patients group (median age 54.5 (21-77), F:M – 13/13). The group consisted of 21 AML-NOS cases, 2 AML cases with inv(16), one case with t(6;9) and one with t(9;11) according WHO classification. ELN cytogenetic risk stratification divided the group into intermediate-1, intermediate-2 and adverse cases in 10, 12 and 4 patients respectively. All patients were treated with Daunorubicin, Cytarabine and Cladribine based first line chemotherapy. We collect bone marrow and blood samples before chemotherapy and blood samples alone 48 hours after chemotherapy start. In all samples leukemic blasts were counted and determined by flow cytometry and the subpopulation of HIF-2 alpha positive blasts was estimated as well. Volunteer bone marrow donors were the control group in this study and the CD34+HIF-2α+ subpopulation was assessed in their bone marrow samples during the routine harvest procedure. The study was approved by the local Ethics Committee.

Results: After the first line chemotherapy 15 patients achieved complete remission (CR-group) and 11 did not (NR group). We did not find significant differences between the groups regarding patients age, the mean percentage of blasts in bone marrow and blood before the treatment, the percentage of HIF-2 alpha positive blasts in BM and blood before and 48 hours after the treatment start (data not shown). But the analysis of the percentage of HIF-2 alpha positive blasts in blood before and 48 hours separately in CR and NR groups revealed quite different dynamics. In CR group the mean percentage of HIF-2 alpha positive blasts was 14.65 (+33.2) and 8.48 (+11.63) before and after chemotherapy respectively (p=NS); in NR group the values were 11.74 (+22.6) and 24.01 (+33.68) respectively (p=0.007) (Figure 1). The Cox analysis revealed HIF-2 alpha positive blasts in blood after chemotherapy to be proportional to death probability (p=0.0036) (Figure 2).

PB1696
RARE BCR/ABL FUSION PROTEINS AND THEIR CLINICAL SIGNIFICANCE INTO PH+ ACUTE MYELOID LEUKEMIACS

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Background: The Philadelphia (Ph) t(9;22)(q34;q11) results in an oncogenic BCR/ABL gene fusion, representing the hallmark of chronic myeloid leukemia (CML), although it has been also described in acute lymphoblastic (ALL) and myeloid (AML) leukaemia. Three main different transcripts have been described (p210, p190 and p230), but rare atypical BCR breakpoints outside the cluster regions have been also reported and their clinical significance is under investigation. Atypical transcript p190 e6a2 is a rare fusion protein associated with aggressive phenotype and dismal prognosis. The breakpoint in BCR intron 6 is responsible for increased kinase activity and greater transforming potential because of the partial loss of the Guanine Exchange Factor (GEF)/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, genetic and analytical 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 performed using the cDNA of the patients.

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 on G-banded metaphases was 46.XY, t(9;22)(q34;q11). While the molecular analysis was negative for the t(9;22)(q34;q11) and showing an e6a2 transcript. The molecular biology analysis revealed the simultaneous presence of the common p190 e1a2 and the rare e6a2 isoforms (Figure 1A). Because of persisted pan-CD34+ and presence of blasts, according to the molecular data, he was then evaluated for a possible t(1;19) and the rare e6a2 isoforms (Figure 1A). Because of persisted pan-CD34+ and presence of blasts, according to the molecular data, he was then evaluated for a possible t(1;19) and the rare e6a2 isoforms (Figure 1A). Because of persisted pan-CD34+ and presence of blasts, according to the molecular data, he was then evaluated for a possible t(1;19) and the rare e6a2 isoforms (Figure 1A). Because of persisted pan-CD34+ and presence of blasts, according to the molecular data, he was then evaluated for a possible t(1;19) and the rare e6a2 isoforms (Figure 1A). Because of persisted pan-CD34+ and presence of blasts, according to the molecular data, he was then evaluated for a possible t(1;19) and the rare e6a2 isoforms (Figure 1A). Because of persisted pan-CD34+ and presence of blasts, according to the molecular data, he was then evaluated for a possible t(1;19) and the rare e6a2 isoforms (Figure 1A).
consolidation chemotherapy was postponed, relapsing without reach the already planned transplantation. At the bone marrow transplantation, the 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 FLT3-ITD mutations.

**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 allogenic 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 allogenic 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 ELSN 2010, 11 were intermediate I and II and 2 were adverse risk. 67% of patients received HMA as second line therapy for their disease, 27% as third line and 6% were beyond the third line. 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 HMA cycles was 2 (range 1-31). 26% of patients underwent allogeneic transplantation after HMA therapy. Median OS was 197 days from the starting of HMA and median EFS was 70 days. Median OS in patients with refractory disease was 91 days and median OS in relapsed patients was 331 days (p=0.0049). Median EFS in patients with refractory disease was 57 days and median EFS in patients in 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 allogenic 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 and arsenic trioxide (ATO) are the current standard treatments in low- and intermediate-risk patients, resulting in significantly high efficacy in Spanish and Italian studies. However, early mortality resulting from coagulation disorders remains high. Furthermore, AraC administration during consolidation is questioned and often limited to high-risk patients.

**Aims:** We aim to compare the efficacy, tolerance and toxicity between 2 consecutive treatment protocols that differed in AraC administration during consolidation.

**Methods:** We studied clinical characteristics, prognostic factors, response to treatment, toxicity, and outcomes in APL patients treated in our Department during the last decade. All patients received induction with AIDA (Idarubicin x4, ATRA until remission) and 2-year maintenance therapy. Protocol 1 included 2 cycles of consolidation with anthracyclines/AraC. Protocol 2 was implemented the last 5 years and included 3 cycles of anthracyclines and AraC only in high-risk patients (PETHEMA LPA2005).

**Results:** APL was diagnosed in 35 patients, of whom 2 patients older than 80 years did not receive treatment and were not included in the analysis. The rest 18 male: 15 female patients aged 37-70(75) years old presented at diagnosis with: thrombocytopenia (32), leukopenia (22), leukocytosis (6), impaired performance status/PS >2 (10), lactate dehydrogenase >400 IU (17), increased d-dimer (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 tolerance 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 leukopenia was significantly higher in Protocol 1 [6(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 reasigned as high or low intensity following evaluation of physician treatment selection. Post-hoc T-tests and Chi-Squared/Fisher’s exact tests were used to determine differences between groups.

Results: Table 1 shows key presenting characteristics of AML patients <60 and ≥60 years 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 were categorized as high risk versus 50% (n=98) of patients ≥60 years of age (high versus low intensity by age group P<0.001). All other patients received low intensity treatment irrespective of age. The most common high intensity treatment given was a cytarabine-based regimen and the most common low intensity treatments were low dose cytarabine-, decitabine- or azacitidine-based regimens.

Table 1. Disease characteristics of patients <60 and ≥60 years of age at diagnosis of AML.

<table>
<thead>
<tr>
<th>Disease characteristics</th>
<th>&lt;60 years old (n=240)</th>
<th>≥60 years old (n=233)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>0.006</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>Leukemia</td>
<td>Others</td>
<td>0.680</td>
</tr>
<tr>
<td>Symptoms</td>
<td>No symptoms at diagnosis</td>
<td>Symptoms (n=24)</td>
<td>0.057</td>
</tr>
<tr>
<td>Performance status</td>
<td>&lt;60% (94)</td>
<td>≥60% (106)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diagnostic tests</td>
<td>No of tests used to establish AML diagnosis</td>
<td>Mean (SD)</td>
<td>0.190</td>
</tr>
<tr>
<td>Diagnostic intensity</td>
<td>&lt;60% (94)</td>
<td>≥60% (106)</td>
<td>0.001</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.

PB1701

A UNIQUE PRESENTATION OF ACUTE PROMYELOCYTIC LEUKEMIA: AORTOILIAC OCCLUSIVE DISEASE (LERICH SYNDROME)

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Background: Acute promyelocytic leukemia (APL), FAB M3 subgroup of acute myeloid leukemia is known for its association with haemostatic disorders. Compared to bleeding thrombosis is a less commonly encountered complication of APL. Thrombosis of major arteries is a rare form of presentation.

Aims: A case, who applied with acute lower limb ischemia and diagnosed with APL and aortoiliac occlusive disease (Leriche syndrome), is presented.

Methods: A 53-year-old female patient presented with weakness, loss of appetite and pain in the lower extremities. She had diabetes mellitus (DM) regulated with metformin, hyperlipidemia (HL), and smoking history. Physical examination revealed general paleness and ischemia around big toe of the right foot. Laboratory studies revealed leukopenia, neutropenia, anemia, thrombocytopenia, elevated D-Dimer. A bone marrow aspiration and biopsy was done to enlighten the etiology of pancytopenia. The pathological examination of the bone marrow revealed abundant granular blasts (78%) and Auer rods. The patient was diagnosed with APL, hypergranular classical form. t(15;17) was positive with fluorescence in situ hybridization. All-trans retinoic acid (ATRA) plus idarubicin treatment was started. In few days symptoms of ischemia progressed and encompassed 2nd, 4th and 5th toes together with the big toe (Figure 1 on the left). Monophasic flow pattern (proximal stenosis?) was detected in bilateral common femoral arteries in lower extremity venous doppler ultrasonography. On CT angiography, abdominal aorta and bilateral common iliac arteries were observed to be occluded from L3 vertebra level till 1.5 cm after aortic bifurcation (Figure 1 on the right). Low-molecular-weight heparin therapy was started. According to rheumatological tests and tests for lupus anticoagu- lant, anticardiolipin and antiphospholipid antibodies, anti-beta-2 glycoprotein-1, protein C-S, Antithrombin III and homocysteine levels, methylenetetrahydrofolate reductase, Factor V Leiden and prothrombin gene mutations no cause of tendency to thrombophilia could be determined. Echocardiography was normal. The patient was transferred to Cardiovascular Surgery Department for axillofemoral bypass operation.

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.

Figure 1.

Results: In APL 80% of thrombotic events occur before treatment or during induction. Acute lower limb ischemia as an initial feature of APL is very rare
which makes our case unique. Thrombotic risk factors in APL include high leukocyte count, presence of coagulation disorder, ATRA+chemotherapy+antifibrinolytic therapy and ATRA syndrome. None of these were seen in the presented case. The effects of known predisposing risk factors to thrombosis meaning DM, HL and smoking cannot be ruled out. But development of acute thrombosis concomitant with APL diagnosis points out to the relationship between these two entities.

Summary/Conclusions: Current literature knowledge is based on case reports and 9 patients with APL who presented with acute lower limb ischemia were reported yet. As far as we know our case is the first APL case presenting with aortoiliac occlusive disease (Leriche syndrome).

PB1702
A CASE OF THERAPY-RELATED ACUTE LEUKEMIA WITH MIXED PHENOTYPE WITH BCR-ABL1 AFTER TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Although therapy-related acute leukemia (tAL) is a well-recognized clinical syndrome and is increasing owing to the prolonged survival of patients treated with chemoradiotherapy, tAL with mixed phenotype is extremely rare.

Aims: Here, we report a rare case of tAL with mixed phenotype with BCR-ABL1 after achieving complete remission (CR) of Diffuse Large B-Cell Lymphoma (DLBCL).

Methods: A 57-year-old woman was diagnosed as DLBCL. The patient received six cycles of R-CHOP regimen with G-CSF injected after each cycle and achieved CR. The patient was readmitted to the hospital after a follow-up examination revealed the presence of immature cells in the blood.

Results: Her complete blood count findings were as follows: hematocrit, 35.1%; hemoglobin, 116 g/L; platelet count, 129×10^9/L; and white blood cell count, 2.41×10^9/L, with 4% blasts, 26% segmented neutrophils, 3% band neutrophils, 39% lymphocytes, and 26% monocytes. Bone marrow aspirations revealed 40.7% blast cells with medium cell size, oval-shaped vesicular nuclei, fine chromatin patterns, and basophilic cytoplasm. On cytochemical staining, these blast cells were positive on PAS and NSE staining, but were weakly positive for MPO staining. Flow cytometric analysis showed that the blasts were positive for both T-lymphoid and myeloid markers (cytoplasmic CD 3,91%; CD 4,84%; CD 8,91%, CD 11b,70% and negative for CD 5,59%; CD 123,0%; CD 34,0%; CD 19,20%; CD 45,58% and CD 68,0%) and negative for CD 2, CD 10, CD 11b, CD 14, CD 15, CD 19, CD 20, CD 65, CD 117, and TDT. Immunophenotyping filled the diagnostic criteria of T/myeloid biphenotypic leukemia based on the scoring system of the EGIL and WHO classifications. Multiplex reverse transcriptase PCR using Hemalign (BioRad Laboratories) revealed the presence of minor BCR-ABL1 (e1a2) fusion transcripts. Chromosome analysis of bone marrow cells revealed because of insufficient mitotic cells. Immunoglobulin heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirates.

Summary/Conclusions: Mixed phenotype acute leukemia is an uncommon subtype that comprises 0.5-1% of leukemia. The T/myeloid phenotype is rarer and represents 35% of all MPAL cases. The risk of secondary malignancies after lymphoma treatment is relatively increased for leukemia. AML, ALL, MDS, CML and chronic myelomonocytic leukemia are reported secondary hematologic malignancies. Until now, only one case of tAL with mixed phenotype after lymphoma has been reported worldwide. To the best of our knowledge, this is the second case of tAL with mixed phenotype after DLBCL. This case is also unique because the BCR-ABL1 has not been described in the literature for patients with tAL with mixed phenotype, after hematologic malignancy. According to the 2016 WHO classification, tAL can be attributed to radiation, alkalyating 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 CHARACTERISTICS OF PATIENTS WITH BLASTIC PLASMOCYTOID DENDRITIC CELL NEOPLASM - DIAGNOSTIC AND THERAPEUTIC DILEMMA
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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a clinically aggressive haematological malignancy that originates from clonal proliferation of plasmacytoid dendritic cells and their precursors. BPDCN is rare, represents less than 1% of acute leukemias. The disease has two patterns of presentations: cutaneous and 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). 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). Hematologic and morphologic findings were assessed by karyotyping and/or reverse transcriptase polymerase chain reaction (RT-PCR). Given the decreasing quality of karyotyping on smears, more centers are now relying on RT-PCR to detect specific translocations. Varying rates of concordance between karyotyping and RT-PCR have been reported and no consensus has prevailed. Given the resource constraint, it is economically non-viable to perform both for prognosis in real world scenarios. Therefore, the cost of the extra tests also adds to the burden of healthcare economy.

Aims: In 132 patients of AML, we aimed at determining the incidence of cytogenetic abnormalities and molecular anomalies detected by Karyotyping and RT-PCR respectively. Concordance rates between conventional cytogenetic tests and RT-PCR were also calculated.

Methods: We conducted a retrospective analysis on the medical records of 132 patients of AML at a tertiary health care facility in India, treated during 2010-2017. Results from commercially available molecular assays for detection of specific translocations by RT-PCR and of adequate samples of karyotype analysis were compared.

Results: In AML patients, out of those tested 50.6% had chromosomal aberrations detected by karyotyping while 30% had a positive detection with RT-PCR. The concordance rate in AML was found to be 56.3%. In a large number (31 in AML) karyotyping provided additional information in the form of detection of deletions, additions and hyper-diploidy (Table 1).

Table 1.

Summary/Conclusions: RT-PCR cannot substitute conventional cytogenetic diagnosis due to the absence of a broad based application for detection of aberrations other than translocations. However, given its efficiency and reliability it can have a complimentary role in prognosis assessment.
Results: Four pts were treated with 3+7 chemotherapy. Complete remission (CR) was achieved in 3 pts, and treatment was continued according to the HIDAC and IDAC protocol. The duration of remission was 3, 8 and 11 months respectively, followed with relapse and fatal outcome. One of the pts died within first 0.5 months after BPDCN was diagnosed. Three pts, treated with Hyper-CVAD, are alive and in CR with duration of 1, 3 and 10 months respectively.

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.

Background: DLBCL is the most common histologic subtype of non-Hodgkin lymphoma (NHL), accounting for about 33% of all NHL cases. However, the healthcare burden associated with DLBCL has not been extensively studied in a US population.

Aims: We evaluated the costs of care and healthcare utilization (HCU) of DLBCL patients treated during routine care in the US.

Methods: The Optum claims database was used to identify adult patients (≥18 years old) with newly diagnosed DLBCL between 01/01/08 and 10/31/15. DLBCL diagnosis was based on ≥1 inpatient claim or ≥2 outpatient claims with DLBCL diagnosis codes, with the index date being the first DLBCL claim. Patients were followed from index date until end of continuous enrollment, death, or end of study period (12/31/15) for the assessment of HCU and costs. DLBCL-related and non-DLBCL-related HCU and costs incurred during follow-up were evaluated. DLBCL-related HCU and costs were medical claims with a primary diagnosis of DLBCL or DLBCL-related treatment (chemotherapy, radiation, stem cell transplant [SCT], supportive care) and pharmacy claims for DLBCL treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPM) costs and reported as mean and standard deviation (SD). Patients with a capitated payment plan were excluded from the cost analysis.

Results: 1,267 treated DLBCL patients were identified. Over the follow-up period, 66.0% of patients had ≥1 inpatient admission, with more patients having a non-DLBCL-related than DLBCL-related admission (Table 1). 60.0% of patients had ≥1 emergency room visit over the follow-up period; visits were predominately for non-DLBCL-related. Nearly all patients had ≥1 physician office visit (92.4%) and other outpatient visits (99.6%). The mean PPPM costs incurred during the follow-up period was $11,890 (SD: $11,515) (Table 1), and costs were higher in Year 1 ($14,402, SD: $10,951) than in Year 2 ($4,190, SD: $8,076). About 55% of costs overall were related to DLBCL medical services ($6,532 PPPM, SD: $6,457). DLBCL-related medical PPPM costs decreased substantially from Year 1 ($8,327, SD: $5,925) to Year 2 ($1,443, SD: $4,349). This decrease was driven by the decreases in chemotherapy and supportive care medical services from Year 1 to Year 2. Non-DLBCL-related medical costs accounted for about 42% of the overall PPPM costs ($4,955, SD: $7,210); and a decrease was observed from Year 1 ($5,840, SD: $7,488) to Year 2 ($2,447, SD: $5,456). Inpatient admission was the main component of non-DLBCL-related costs, and associated costs decreased from Year 1 to 2.

Table 1.
Summary/Conclusions: The economic burden associated with the treated DLBCL population is high, with the majority of costs incurred during the first year of diagnosis. Between the first and second year of diagnosis, costs decrease mainly because of the decrease in the DLBCL-related treatment costs. In addition, HCU for DLBCL-related services decreased in Year 1 vs Year 2.

PB1706
PHARMACOKINETICS OF RITUXIMAB IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Rituximab dosing is based on evidence from clinical practice rather than from consideration of pharmacokinetics and factors influencing individual exposure. Clinical use of rituximab can be improved through a more individualized treatment.

Aims: The objective of this investigation was to typify rituximab pharmacokinetics in 29 newly diagnosed patients with the diffuse large B-cell lymphoma who received rituximab in combination with cyclophosphamide, doxorubicin, vincristine and methylprednisolone every three weeks. The association of rituximab pharmacokinetics with clinical outcome was also investigated.

Methods: Rituximab serum levels were defined by enzyme-linked immunosor- bent 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 0.206 vs 0.238 l/h (range 0.18-0.292 l/h and 0.47-0.390 l/day, correspondingly) and corresponding 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, while in patients with disease progression it was 0.22% 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.

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 have been investigated 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 63 (range 20–87) were included. Patients were 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 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 71 (18.1%) and 124 (31.6%), respectively.

Conclusion: Pretreatment albumin level was 51.2 g/l (range 20–51.4 g/l), platelet level was 274x10⁹/l (range 50–84x10⁹/l), C-reactive protein was 10.2 mg/l (range 0.1–438 mg/l), etrythrocyte sedimentation rate (ESR) was 30 mm/h (range 2–636 mm/h), and albumin level was 38 g/l (range 20–51 g/l). Complete remission (CR) was achieved in 288 patients (73.3%), partial remission (PR) in 58 (14.8%), stable disease (SD) in 5 (1.5%) and progressive disease in 42 (10.7%). Disease relapse was confirmed in 59/348 patients (17.0%). OS was influenced by the presence of B symptoms (p=0.004, 95% CI 1.263-4.832), low albumin level (p=0.0001, 95% CI 1.601-3.883), and albumin level (p<0.0001, 95% CI 0.905-0.953). Optimal cut off point for albumin level was 34 g/l, and was determined by Receiver operating characteristic (ROC) curve (AUC 0.699, 95% CI, 0.629-0.770, p<0.0001). The prognostic value of IPI was highly statistically significant for OS (p<0.0001, 95% CI, 1.545-2.236).

Summary/Conclusions: Although albumin is widely used as a prognostic index in DLBCL, it cannot fully recognize high-risk patients. Pretreatment albumin level may represent a useful tool in order to discriminate high-risk patients and is likely to add significant information to the IPI.

PB1709
TREATMENT PATTERNS AND TREATMENT RESPONSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY
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Background: DLBCL is the most common histologic subtype of non-Hodgkin lymphoma. Treatment guidelines recommend rituximab in combination with chemotherapy as first-line therapy (1LT). For patients who are refractory or relapse, high-dose chemotherapy with stem cell transplant, combination chemotherapy, or single-agent rituximab are recommended in subsequent lines.

Aims: To compare real-world treatment patterns of patients with newly diagnosed DLBCL to NCCN guideline recommendations.
TP53 GENE MUTATIONS IS A PREDICTOR OF HIGH GRADE B-CELL LYMPHOMA PROGRESSION
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Background: High grade B-cell lymphoma (HGBL) is subdivided on poor prognosis double-hit (DH) and not otherwise specified (NOS) variant, which appears sometimes with primary refractory behavior. Mutations in TP53 gene (MUT-TP53) lead to blockage of apoptosis in cells and appearance of additional oncogenic events contributing to tumor progression. Correlation between presence of MUT-TP53 and anti-tumor response in patients with HGBL is unclear.

Aims: To evaluate an effect of MUT-TP53 on survival parameters of patients with high grade B-cell lymphoma.

Methods: Since 2005 to 2017 years in FGBU National Research Center for Hematology Russian Federation diagnosis of high grade B-cell lymphoma were established in 47 patients: 13 – double hit, 34 – NOS. Most patients received rituximab in combination with chemotherapy. A small proportion of patients received single-agent chemotherapy. In DH, rituximab (12.6%) remained the top single agent used, while bendamustine+ruxitumab (15.7%) and R-CHOP (8.2%) were the most common combinations; 82% of patients received stem cell transplant. Median (IQR) duration of 2LT was 2.1 (1.2–3.8) months. Of the 2LT patients, 44.0% (n=70) had evidence of remission, 26.4% (n=42) progressed, and 3.1% (n=5) had no evidence of remission. 34 patients who progressed after 2LT received third-line therapy (3LT); 29.4% received a single agent, while 70.6% received combination chemotherapy. In 3LT, rituximab (5.9%), etoposide (5.9%), and carboplatin (5.9%) were the most common single agents, while bendamustine+ruxitumab (20.8%) and etoposide+carboplatin+ruxitumab (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: In high grade B-cell lymphoma: 1) MUT-TP53 is an independent factor of early disease progression in high grade B-cell lymphoma.

PB1711
HTLV-1 INFECTION INCREASED THE RISK OF OTHER MALIGNANCY
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Background: The correlation between HTLV-1 infection and malignant neoplasm other than ATL remains unknown. Some previous studies have indicated that the frequency of primary malignant neoplasms in patients with HTLV-1 seropositive is higher than HTLV-1 seronegative.

Aims: To clarify the correlations between HTLV-1 infection and malignant neoplasms other than ATL.

Methods: We retrospectively analyzed 203 patients with HTLV-1 seropositive who were diagnosed between 2006 and 2015 at Kansai Medical University Hospital.

Results: Among 203 patients (median age 62 years: range 19 to 86 years), 43% was carrier and 57% was diagnosed with ATL. According to clinical subtype, 5% was chronic, 38% was smoldering, 28% was acute, 29% was lymphoma type. Median overall survival was 30 months in carrier, 10 months in acute, 8 months in lymphoma, and smoldering was not available. In all HTLV-1 seropositive patients, the occurrence of primary malignant neoplasm was 32%, they were all carrier or smoldering. Among them, 53% was hematology malignancy (T cell lymphoma; 41%, B cell lymphoma; 29%, MPN; 18%, MDS, 12%), Solid tumor was 47% (lung cancer; 33%, prostate cancer 13%, colon cancer; 13%, renal cell cancer; 13%). Four patients with HTLV-1 carrier who developed primary malignant neoplasm received standard chemotherapy for the neoplasm, and after the chemotherapy they developed 3 acute type and 1 smoldering type ATL.

Summary/Conclusions: In our cohort, the occurrence of primary malignant neoplasm with HTLV-1 seropositive patients was significantly high. Chronic HTLV-1 infection might associate with reduction of cytotoxic T cells and an increased risk of developing other malignancy. Furthermore, cytotoxic chemotherapy for primary malignant neoplasm might reduce cytotoxic T cells for HTLV-1 and exacerbate ATL conditions.

PB1712
THIOTEPA BUSULFAN CYCLOPHOSPHAMIDE, A TOXIC CONDITIONING FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CENTRAL NERVOUS SYSTEM LYMPHOMA: REMISSION OR INFECTION?
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Madrid, Spain, June 22 – 25, 2017
Background: CNSL represent 4% of central nervous system (PCNSL) and secondary CNS lymphoma (SCNSL) occur in 7% of systemic lymphoma. Overall survival (OS) and progression free survival (PFS) of these patients have dramatically increased in PCNSL since the introduction of Methotrexate high doses and ASCT usually conditioning with TBC (Thiotepa, Busulfan and Cyclophosphamide). The studies usually tend to recommend TBC/ASCT in front line for patients under 65 years old without CNSL with very few prospective data about this strategy.

Aims: We report in this multicenter retrospective study our experience concerning TBC/ASCT and its main toxicities.

Methods: All patients treated with TBC/ASCT for PCNSL or SCNSL from August 2010 to November 2016 in our centers were researched by using CHIMIOS software. All patients treated with TBC consisted Thiotepa (250mg/m² from d-9 to d-7), Busulfan (3.2mg/kg/d from d-6 to d-5 and 1.6mg/kg/d on d-4) and Cyclophosphamide (60mg/kg/d on d-3 and d-2) followed by ASCT (transplantation at d0). Clinical data were extracted from the medical records. We measured OS and PFS from the date of ASCOT and transplant related mortality (TRM) (defined by death occurred 3 months after ASCT).

Results: 24 patients, without any major comorbidity, were included. Median age at ASCOT was 58 years (23-66). 22 of 24 were DLBCL and 2 follicular lymphoma and there were 15 PCNSL and 9 SCNSL. All but one, received 1 or 2 lines of chemotherapy (with high doses Methotrexate in first or second line) before ASCOT. 15 were in complete response (CR) and 9 in partial response (PR) before TBC/ASCT. Median duration of hospitalisation was 33 days (15-78 d) and of aplasia was 14 days (7-37 d). Median follow-up was 10 months (0-73). At the end of follow up 5 patients have died. Among the 3 patients older than 60 years of age at ASCOT, 1 died 6 months after ASCT. 12 patients treated with R-CHOP and PFS were respectively 78% and 73%. Surprisingly (Table 1), we noted an important rate of toxicity (100% with 66% ≥grade 3) with 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 fungall infections (3 candida, 1 aspergillus and 1 cryptococcus).

Table 1.

We observed 5 deaths (4/5 older than 60 years) in first 3 months due to a septic choco, 4 associated with a persistent coma and 2 with an acute respiratory distress syndrome.

Summary/Conclusions: To our knowledge, here is one of the biggest retrospective cohort concerning TBC/ASCT in CNSL. If TBC seems to give interesting response rates (72% CR), we noted an unacceptable toxicity compared to other used conditionnings (for example TRM with Thiotepa Carmustine is 4% and high TRM=21% in this group). The comparison of different regimens is needed to confirm this result and to validate this regimen in the independent cohort.

PB1714

TREATMENT RESULTS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA FROM HIGH RISK AND HIGH-INTERMEDIATE RISK GROUPS I. Kriačokh1,2, K. Filipenko2,2, A. Martynychk2,2, I. Titenenko2,2, I. Stepanishyna2, O. Aleksy2,2, I. Dyagi2,2, E. Kushchevych2,2, Z. Martina3, V. Kozlov4, O. Aleksyk2, I. Dyagil3, E. Kushchevyy2, Z. Martina3, V. Kozlov4

Background: Using of Rituximab-containing regimens, as the «gold standard» of survival and progression free survival (PFS) of DLBCL, showed significant improvement in the treatment results throughout all prognostic groups. The "real-life" treatment approaches vary depending on financial support of healthcare system in different countries. Unfortunately, treatment results in patients with DLBCL from high and high-intermediate risk groups are still unsatisfying.

Aims: Aim of our study was to compare efficacy and toxicity of different treatment approaches in patients with DLBCL from high risk and high-intermediate risk groups.

Methods: Prospective cohort study was initiated in 2014 in three Ukrainian centers. Patients with newly diagnosed DLBCL and ≥3 risk factors according to International Prognostic Index (IPI) were treated according to "investigators decision" (ID). The "real-life" treatment approaches in patients with DLBCL from high and high-intermediate risk groups. Other risk factors included age, performance status (PS) and other variable interacting with IPI.

Results: 104 patients were included into analysis in January 2017, 50 males (48.1%), 54 females (51.9%), in the age 23-86 years old, median age 63 years (95% CI 60-65). Observation period was 1-64 months, median – 10.5 months. Patients were divided into three groups according to the treatment regimen. Patients treated with CHOP-like regimens were included into the first group (53 patients, 51.0%). Patients treated with R-CHOP were included into the second group (40 patients, 38.5%) and 12 patients (12.5%) treated with R-DA-EPOCH were included into the third group. Significant difference between the groups was observed by the age (younger patients in the 3rd group, p=0.042) and stages distribution (early stages were more common in the 1st group, p=0.05).

We observed 5 deaths (4/5 older than 60 years) in first 3 months due to a septic choco, 4 associated with a persistent coma and 2 with an acute respiratory distress syndrome.

Summary/Conclusions: The level of ORR and CRR was significantly higher in the 2nd R-CHOP group. 2-year PFS was significantly higher in the R-DA-EPOCH group, as well. There was no significant difference in the level of 2-year OS between the groups. Toxicity was acceptable in all groups. Levels of neutropenia, febrile neutropenia and cardiotoxicity were less common and neutro-toxicity was more frequent in the R-DA-EPOCH group. Thus, R-DA-EPOCH could be considered as the most efficient treatment regimen in patients with DLBCL from high and high-intermediate risk groups.

PB1715

PROGNOSTIC MODEL WITH NEUTROPHIL-LYMPHOCYTE RATIO AND PERFORMANCE STATUS IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP S.-I. Go1, G.-W. Lee1

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 treatment response (CR+PR) in NLR groups were compared using 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.

PB1716

HIGH LEVEL SERUM LEVELS OF SOLUBLE INTERLEUKIN-2 RECEPTOR ARE ASSOCIATED WITH A POOR PROGNOSIS IN CASES OF RELAPSED/REFRACTORY PERIPHERAL T CELL LYMPHOMA, NOT OTHERWISE SPECIFIED M. Morita1, K. Ishikawa1, A. Kato1, A. Tanaka2, A. Nakamura1, A. Fujimoto1, T. Yabushita1, Y. Shirouzu1, Y. Ono1, A. Hashimoto2, N. Hiramoto1, S. Yoshioka1, N. Yonetani1, Y. Tanaka2, A. Matsushita1, H. Hashimoto1, I. Sinzato2, T. Iwashita1

Background: The prognosis is extremely poor for cases of relapsed/refractory peripheral T cell lymphoma, not otherwise specified (PTCL-NOS), and there was a significant survival advantage between patients achieving a CR and others. Our results, however, showed that high NLR was clearly 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: 260–24,400 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 (≥H-I) 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 multivariable analysis was performed using the following factors: serum sIL-2R levels (high vs low), secondary IPI (≥H-I vs ≤0), age, and sex. The results are shown in 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 LARGE B-cell LYMPHOMA (LB-L)

**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 patients were available for follow-up. A total of 28 lymphoma patients with pre-existing autoimmune diseases were finally analyzed. For the further comparison, 56 lymphoma patients without pre-existing autoimmune diseases who were adjusted for age and gender were considered to be the control group. Response rate, progression-free survival (PFS), and overall survival (OS) were compared between these two groups of patients.

**Results:** Rheumatoid arthritis was the most common autoimmune disease in 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±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: 0.672–2.601, 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.

**PB1718**

**THE DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS OF CIRCULATING MiRNA-21 IN A SAMPLE OF HEPATITIS C/NONE HEPATITIS DIFFUSE LARGE B-CELL LYMPHOMA EGYPTIAN PATIENTS**

**Background:** MicroRNAs (miRNAs) are small RNA molecules which control the expression of many target messenger RNAs involved in cell differentiation, proliferation and apoptosis. Circulating microRNAs are potential biomarkers of diagnostic and prognostic impact in various inflammatory and malignant diseases. Until now, no studies evaluated the diagnostic and prognostic impact of circulating miRNA-21 in diffuse large B-cell lymphoma (DLBCL) patients.

**Aims:** Linking inflammation with malignancy, we studied the expression of miRNA-21 in sera of hepatitis-C virus and none hepatitis DLBCL patients, aiming to identify its differential expression and prognosis in DLBCL with its subtypes; germinal center B-cell (GCB) and activated B-cell-like (ABC) and to evaluate its relation with HCV.

**Methods:** MiRNA-21 expression was measured using Taq-Man quantitative RT-PCR in sera of 30 newly diagnosed DLBCL patients (HCV positive (n=10), HCV negative (n=20)) and 20 controls (HCV positive (n=10), HCV negative (n=10)). The diagnosis of DLBCL and its sub-classification in GCB and ABC subtypes were done by applying the criteria of the WHO classification of tumors of the hematopoietic and lymphoid tissues 2008 and revised in 2016. HCV was confirmed by Immunohistochemistry using antibodies to CD10, BCL-6, MUM-1 and BCL-2. HCV was diagnosed by detection of anti-HCV antibodies in sera of patients and controls by Enzyme-Linked Immunosorbent Assay (ELISA) technique and HCV genetic detection and quantification by polymerase chain reaction (PCR). All the patients received CHOP chemotherapy and were followed up for an average of 24 months.

**Results:** MiRNA-21 expression was significantly higher in DLBCL patients than in controls (p<0.00). Significant positive correlations between miRNA-21 and LDH, IPI, disease stage were detected (p<0.05). Significantly higher miRNA-21 levels were detected in ABC subtype compared to GCB subtype (p=0.00). Significantly higher miRNA-21 expression levels were detected in BCL6 negative, CD10 negative, MUM1 positive DLBCL cases compared to its levels in BCL6 positive, CD10 positive and MUM1 negative cases, (p=0.018, 0.002 and 0.001 respectively). Higher miRNA-21 was associated with worse response (p=0.016), 2-year overall (p=0.017) and 2-year progression free survival with statistical significance (p=0.003). Significantly higher miRNA-21 levels were detected in HCV positive DLBCL patients compared to HCV-negative patients (p<0.00). Higher miRNA-21 levels were detected in HCV positive ABC subtype than GCB subtype (p=0.05). Significantly higher levels were also detected in HCV positive controls compared to HCV-negative controls.

**Summary/Conclusions:** Our study showed that miRNA-21 was overexpressed in DLBCL patients, displaying higher levels in ABC than in GCB subtypes. MiRNA-21 was associated with poor response to treatment and survival in DLBCL. According to our results, miRNA-21 is a potential marker of necro-inflammation independent of its role in tumorigenesis, showing higher expression in HCV positive DLBCL patients compared to none hepatitis patients.

**PB1719**

**A NEW SCORING SYSTEM FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA – A RETROSPECTIVE MULTI-CENTER ANALYSIS IN TAIWAN**

**Background:** The incidence of primary central nervous system (PCNSL) lymphoma remains high worldwide, with different geographic distribution. This disease affects patients usually with high-intermediate to high risk of non-Hodgkin lymphoma and its outcome is very poor. The treatment for PCNSL includes local radiation therapy and methotrexate. Several scoring systems have been developed for PCNSL. In Taiwan, we developed a new scoring system using a multivariate analysis from a large multi-center study.

**Methods:** We retrospectively reviewed medical records of 141 patients with PCNSL from 11 centers in Taiwan from January 2010 to December 2015. The median age was 57 years (range 17–88 years). The median follow-up was 4.5 years (range 0–116 months). The disease-free survival was analyzed using the Kaplan-Meier method. The univariate analysis was performed using the log-rank test. The multivariate analysis was performed using the Cox proportional hazards model.

**Results:** The median age at diagnosis was 65 years (range 17–88 years). The median follow-up was 3 years (range 0–116 months). The median survival was 10 months (range 0–116 months). The 1-, 2-, and 5-year survivals were 44%, 25%, and 25%, respectively. The univariate analysis showed that the Karnofsky performance status (KPS), the number of sites involved, and the type of initial therapy were significant predictors of survival. The multivariate analysis showed that the Karnofsky performance status (KPS), the number of sites involved, and the type of initial therapy were independent predictors of survival. The new scoring system for PCNSL was developed using a multivariate analysis of the significant predictors. The new scoring system for PCNSL was developed using a multivariate analysis of the significant predictors. The new scoring system for PCNSL was developed using a multivariate analysis of the significant predictors. The new scoring system for PCNSL was developed using a multivariate analysis of the significant predictors. The new scoring system for PCNSL was developed using a multivariate analysis of the significant predictors.
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) (JCO 2002;21:266).

Neither the IELSG nor MSKCC scoring system is ideal to distinguish the 2-year OS of the PCNSL patients in Taiwan. The new scoring system comprising age □ 60 years and ECOG PS □ 2 seemed to provide a better prognostic power for Taiwanese patients.

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 G-CSF prophylaxis in neutropenic fever.

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 NCI-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 had neutropenia prophylaxis had an NF episode. The Odds ratio (OR) for the patients under prophylaxis was 0.232 (CI 95%; 0.085-0.634, p=0.004) (Figure 1).

Figure 1.

Summary/Conclusions: The OS and the PFS in our sample is similar as described in larger studies. The days of admissions adjusted to the CIRS scale gave us a tool to help physicians to discriminate patients who will have prolonged admissions when treated with the standard of care. The CIRS scale also help separate two distinct OS curves, giving physicians a new tool to help discriminate worse prognostic patients, making them good candidates for adapted therapies. The use of GSCF prophylactic can protect the elderly patients from NF, and should be used in all patients in this category.

PB1721

PRIMARY ADRENAL LYMPHOMA: A SINGLE-CENTER EXPERIENCE

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Background: Primary adrenal lymphoma (PAL) is rare, with slightly more than 250 cases currently described in the English-language literature. In current classifications, there is not yet a consensual definition of PAL. The aim of this study is to report a large single-center clinical case series of primary adrenal lymphoma (PAL) in terms of clinical presentation, pathological and imaging features, and treatment outcome.

Methods: We performed a retrospective analysis of 21 patients diagnosed with PAL who presented to our center between January 2005 and January 2017.

Results: Median age at presentation was 48 years (range: 27–73) with a male-to-female ratio of 6:2. Bilateral and right-sided adrenal involvement were seen in 12/21 and 7/21 patients, respectively. Adrenal insufficiency (AI) was seen in

PB1720

RELEVANCE OF CIRS SCALE IN THE PROGNOSIS OF DIFFUSE LARGE B CELL LYMPHOMA IN ELDERLY PATIENTS

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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 regime. Patients that relapse can be rescued with salvage treatment in 20-30%. The elders are not considered for full standard treatment in many centers. Geriatric scales are starting to being 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 G-CSF prophylaxis in neutropenic fever.

Methods: We performed a retrospective analysis of 21 patients diagnosed with PAL who presented to our center between January 2005 and January 2017.

Results: Median age at presentation was 48 years (range: 27–73) with a male-to-female ratio of 6:2. Bilateral and right-sided adrenal involvement were seen in 12/21 and 7/21 patients, respectively. Adrenal insufficiency (AI) was seen in
6/10 evaluated patients. Computed tomography scans showed slight to moderate contrast enhancement of adrenal masses in 4/5 patients (80%), and magnetic resonance imaging identified a normal T1 and longer T2 phase. Diffuse large B cell lymphoma (DLBCL) was the most common immunophenotype (82.6%). Two patients died due to rapid disease progression before treatment. Three patients were treated with chemotherapyeextemal beam radiotherapy. Two patients received autologous stem cell transplantation as consolidation therapy. Five-year overall survival and progression-free survival were 54.2% and 51.0%, respectively.

Summary/Conclusions: These findings suggest that PAL should always be considered in differential diagnosis of adenal mass with AI. Moreover, DLBCL was observed as the most common histological subtype of PAL. Despite the contrasting previous reports, long-term prognosis of PAL is not necessarily inferior to that of non-Hodgkin lymphoma in general.

PB1723
HEMATOLOGICAL MALIGNANCIES IN SOLID ORGAN TRANSPLANT RECIPIENTS: RETROSPECTIVE SINGLE-CENTER ANALYSIS IN JAPAN
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Background: The aim of this study was to identify the incidence, characteristics, risk factors and prognosis of HMs in solid organ transplant recipients at our institution.

Methods: Clinical data of patients undergoing kidney, liver and heart transplantation at Hokkaido University Hospital between January 1965 and December 2015 were retrospectively reviewed. Kaplan-Meier analysis was performed for the cumulative incidence rates (CI) of HMs, graft survival and patient survival. Patient’s characteristics were compared between groups by the student t-test or K–square test.

Results: A total of 16 cases of HMs were identified. 9 post-transplant lymphoproliferative disorder (PTLD), 5 acute myeloid leukemia (AML), 1 myelodysplastic syndrome (MDS), 1 myeloproliferative neoplasm (MPN) and 1 recurrent non-Hodgkin lymphoma. The CI of PTLD were 1.1%, 1.5% at 10 years in kidney transplant recipients (n=352), 0.92%, 2.6% at 5, 10 years in liver transplant recipients (n=287) and 29% at 1 year heart transplant recipients (n=5), respectively (P<0.0001). AML/MDS and MPN were more frequent in liver transplant recipients, and CI were 2.3% at 5 and 10 years (P<0.01). There was no difference in background factors other than transplant organ type between recipients with HMs and without HMs. Patients with EBV-positive PTLD (n=5) were younger (P<0.05) and had less extranodal diseases (P<0.05) compared with EBV-negative PTLD (n=4). All patients with monomorphic PTLD (n=4) were treated with chemotherapy combined with rituximab and had been in remission. In patients with other PTLD, reduction or withdrawal of immunosuppressant or rituximab alone resulted in stable disease or remission. All AML/MDS but 2 acute promyelocytic leukemia in pediatric patients were chemo-refractory and lethal. 2100-day OS were 92% and 100% in kidney and heart transplant recipients. In liver transplant recipients, 10-year OS were 74%, 100% and 50% in patients without disease, with PTLD and with myeloid neoplasm, respectively.

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 the awareness of the 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
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Background: Cytogenetic abnormalities of MYC are associated with poor prognosis in patients with diffuse large B-cell lymphoma (DLBCL). In the Authors’ experience, rearrangement of MYC reportedly occurs in approximately 10% of DLBCL cases. In addition, in various clinical trials of rituximab with standard dosing, female receiving rituximab had better outcomes than male. However, gender-segregated outcomes of patients with MYC rearrangement have not been reported. In addition, the gender segregation of known prognostic factors, such as high international prognostic index (IPI) score, elevated lactate dehydrogenase (LDH) level, poor Eastern Cooperative Oncology Group performance status (PS), advanced stage, and ≥2 extranodal sites, not yet been fully elucidated.

Aims: The aim of this study was to determine the gender segregation of clinical factors and gender-specific prognostic factors, including MYC (fluorescence-in-situ hybridization: FISH) in patients with DLBCL by analyzing data from consecutive DLBCL patients.

Methods: In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. Cytogenetic analysis and FISH were performed in 318 patients. The median age was 70 years (range: 27–92 years). The median follow-up was 17 months (range: 1–81 months). To adjust the impact of age, LDH level, PS, stage, ≥2 extranodal sites, IPI, COO, BCL2 (IHC), BCL6 (IHC), MYC (IHC), double expressor (IHC), MYC (FISH), and other significant factors, uni-

PB1721
EFFICACY AND SAFETY OF IBRUTINIB THERAPY IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA IN REAL-LIFE – A MULTICENTRIC STUDY (R.E.-A.PULIAN HEMATOLOGY NETWORK)
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Background: Malignant lymphoma (MCL) is a rare subtype of non-Hodgkin lymphoma that has an aggressive clinical course and poor prognosis. Although current front-line combination chemo-immunotherapies followed by autologous stem-cell transplantation (ASCT) have improved the outcomes of affected patients (pts), the role of ibrutinib in the context of MCL, an oral covalent inhibitor of Bruton tyrosine kinase that showed significant activity in relapsed/refractory MCL in clinical trials, but in real-life routine, the efficacy and safety may not always mirror those seen in clinical trials.

Aims: To investigate the clinical use of ibrutinib as a single-agent in 31 pts with relapsed or refractory MCL to obtain additional information about predictive factors, outcomes and toxicity in a real-life context.

Methods: We studied a group of 31 pts treated (or still in treatment) with ibrutinib to assess effectiveness in terms of overall response rate, complete response rate, progression free survival and adverse events (AEs) in a real-life context. Data were collected also with reference to clinical and biological characteristics of the disease (MIPI, MIPIb, bone marrow involvement, stage, histology, presence of bulky mass and/or extranodal disease) both at the time of diagnosis and at the time of the start of ibrutinib therapy, and to the type and number of previous therapies.

Results: 100% of pts with ibrutinib therapy, the median age was 70 years (range, 45-82), 100% of pts had high risk MCL according to the IPI score, 45.2% of pts presented extranodal involvement of MCL. 26 pts were treated for relapsed MCL, 5 for refractory disease. They had received a median of 2 (range 1-5) prior therapies including different chemo-immunotherapy schemes. ASCIT and newer agents such as bortezomib, lenalidomide, tisilumab. We observed 6 complete responses, 1 after only 2 months of therapy, the others within 6 months of therapy. After 15 months, we observed 4 relapses, characterized by leukemic disease. In 13 pts we encountered central nervous system involvement, and 8 progression. 80% of pts treated for refractory disease presented progression within 6 months. 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). The most common hematologic event observed was neutropenia (9.7% of pts, grade ≥ 2). With an estimated median follow-up of 6 months (range, 4-29), 19 pts are still receiving treatment, 12 have discontinued therapy for relapse or progression of disease. Follow-up is still ongoing.

Summary/Conclusions: Single-agent oral ibrutinib shows a high response rate and produces rapid responses regardless of the number and quality of prior treatments. However, the quality of time on response does not appear 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 pts suggests that ibrutinib may have an anabolic effect, including alteration of body pressure and lipid profile. Larger cohorts of pts and longer follow-up are warranted to confirm these preliminary data.
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. Multivariable analyses were then performed using these factors in the Cox proportional hazard model. MYC rearrangement (FISH) [hazard ratio (HR): 9.13, 95% confidence interval (CI): 2.33–35.77, P=0.0015] and IPI ≥3 were identified as independent significant prognostic factor for OS in the female patients with DLBCL. Furthermore, MYC rearrangement (FISH) [HR: 2.47, 95% CI: 1.87–327.8, P=0.01494] and elevated LDH level were identified as independent significant prognostic factor for PFS in the female patients with DLBCL. On the other hand, PS ≥2 was identified as the only significant prognostic factor for OS (HR: 44.27, 95% CI: 6.71–292.2, P=0.001), but not for PFS in the male patients with DLBCL. Five of seven female patients with DLBCL and MYC rearrangement died from lymphoma progression. The median OS in the female patients with DLBCL and MYC rearrangement was 8.0 months (range: 1–35 months) compared to 21.5 months in those without MYC rearrangement (range: 1–79 months, P=0.003). On the other hand, in the male patients (n=13) with DLBCL, MYC rearrangement was not significantly associated with poor OS (Figure 1).

**Summary/Conclusions:** These results suggest that MYC rearrangement by FISH is significantly associated with very poor OS and PFS in the female patients with DLBCL but not the male patients with DLBCL. On the other hand, PS ≥2 is significantly associated with poor OS in the male patients with DLBCL.

**PB1725**

**ASSESSING THE RISK FOR PERFORATION IN DIFFUSE LARGE B-CELL LYMPHOMA INVOLVING THE INTESTINES USING COMPUTED TOMOGRAPHY CHARACTERISTICS.**

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**Background:** About 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 most frequent (61%), followed by large intestine and ileocecum (23 and 16%, respectively). Forty-three (88%) patients underwent CT scan at diagnosis. 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 transmural lesion (p=0.008, HR=1.06, CI 1.071–1.166). Each extra centimeter to the length of the GI segment involved was associated with a 6% increase in the risk for perforation. There was no association between sex, age, performance status, hemoglobin, LDH, albumin, iron, ferritin, Ki67, disease stage, anatomical location nor the involved site wall thickness and risk of perforation.

**Summary/Conclusions:** DLBCL patients presenting with an involvement of a long intestinal segment, especially with a concentric, transmural lesion, are at higher risk for perforation. These patients should be considered for a preemptive surgical resection, dependent on lesion site and operative risk.

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**Figure 1. Overall survival.**
The incidence of double or triple hit lymphomas in our institution is consistent with the literature. 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 chemosensitive 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 (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. 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 (68%), leptomeningeal infiltration 4 (13.8%), high LDH 19 (65.5%), IPI 3-5 21 (72.4%). Salvage therapies used were: R-EHAP 23 (79.4%), R-ICE 1 (3.5%), MTX-ARAC 4 (13.8%) in patients with leptomeningeal infiltration and intensive burt-kitt-like therapy 1 (3.4%) in a double hit patient. Twelve (41.4%) did not complete the planned doses (10% for 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 one progressed with a median follow-up of 5 months. One patient was rescued with a third line of treatment (R-ICE) and allogeneic transplant, and he 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).

Table 1.

Summary/Conclusions: Patients with DLBCL refractory to first line R-CHOP are not rescued with current salvage therapies, and in this setting DLBCL must be considered an incurable disease with a very short survival, similar to that of patients treated with palliative care. Patients with B symptoms and elevated LDH at diagnosis have a significant higher risk to be refractory to R-CHOP. It is imperative to identify early these patients and to design new therapies for them.
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 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 CSF clearance. Short follow up precluded assessment of cumulative incidence of CNS relapse/progression.

Results: Data from 36 patients were analysed. A total of nine patients were considered to have CSF involvement, all of them detected by FC. Of note, all of them were considered negative for CSF infiltration by standard cytology. Three additional patients were administrated as therapy as LCB, none of which MR imaging revealed any 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). CSF clearance (AE) 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 reoccurrence during the IT treatment. The overall AE incidence was 66.7%. The most common AE include: headache, peripheral sensory neuropathy, back pain and nausea. Severe neurotoxicity has been encountered in four patients: cauda equina syndrome (2), encephalitis (1) and cauda equina syndrome (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 were manageable with time.

Summary/Conclusions: The use of liposomal formulation of cytarabine for IT administration has become an effective option for the treatment of leptomeningeal involvement by haematological malignancies. Neurological AE are reversible; however, they accumulate and worsen with time, thus precluding long-term use.

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 (2). Over 40% of patients with DLBCL are above the age of 70, and the comorbidities in this age-group present significant challenges and complexities with regards to selecting and implementing treatment regimens (2).

Aims: We present a retrospective analysis of outcomes for patients with high-grade DLBCL (stage 3 or 4 disease) who have received fewer than 6 cycles of full-dose R-CHOP or R-GCVP because of poor tolerability or disease progression with treatment.

Patients and Methods: Retrospective data were collected from the cancer registry for all newly-diagnosed DLBCL patients who received R-CHOP or R-GCV chemotherapy, with data collected from Jan 2010 to Feb 2017 from Ipswich Hospital NHS Trust, United Kingdom. Patients who completed 6 cycles of chemotherapy were excluded. Interim PET-CT scan/staging CT scan was done to assess the disease response to therapy after 2 cycles of chemotherapy. The main baseline characteristics collected were age, sex, ECOG Performance Status, Ann-Arbor Stage and IPI risk stratification. The primary end point was progression free survival (PFS) from time of diagnosis. Secondary endpoints were overall response rate (ORR), PFS from time of diagnosis, and causes of death.

Results: Out of 87 patients, 35 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 0-2 in 10 (83%) and ≤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 12 patients. 11 patients received R-CHOP and 1 patient received R-GCVP. The median length of treatment was 3.5 months (range: 1-9 months). The overall response rate was 50% of patients in 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 was 12 months (range: 1-52 months) and the median overall survival of living patients was 12 months (range: 1-45 months). The most common reasons for stopping the treatment were intolerance of side-effects (4 out of 12) or neutropenic sepsis (3 out of 12). 2 out of 12 patients received an incomplete course of chemotherapy due to non- response or progression of disease with treatment.

Conclusions: DLBCL treated with less than 6 cycles of full dose R-CHOP or R-GCVP chemotherapy may achieve sustained long-term remission in selected patients with high IPI and significant comorbidity. 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 poly-morphism of folate pathway genes and/or of NF-kb, which have been previously suggested as plamaco-genomic targets in lymphoid neoplasms. A risk stratification model needs to be developed to reduce drug toxicity and other short and long term treatment related complications so as to improve patient experience, and pharma-economic benefit.

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 diagnosis, synchronous and metachronous type are defined as diagnosis into 6 months, or more 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, quadple neoplasms 2 cases. The median age was 7 years (ranged 51-93yrs), the synchronous type 70yrs(ranged 51-88yrs), the metachronous type was 73yrs(ranged 57-93yrs). 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, esophageal cancer 9 cases, hepaticocellular carcinoma 12 cases. In double neoplasms 149 cases, metachronous type was 112 cases. The median age of first diagnosis, 68yrs(ranged 43-85yrs), the second cancer were 74yrs(ranged 57-89yrs). About interval between solid cancer and NHL, median interval time was 58M, solid cancer precence case was 53 cases, interval was 81M (ranged 7-564M), hematological malignancy precedence case was 59 cases interval was 55M (ranged 8-364M). 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 18M (ranged 1-211M), synchronous type 14M(ranged 2-132M), metachronous type 22M (ranged 1-116M).

Summary/Conclusions: In the case of a double cancer including solid cancer and NHL, the first cancer occurs in elderly. Diagnosis of malignant neoplasms at young age is rare and in 419 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 formalignant neoplasms. We think that a prognosis is improved.

PB1732

RETROSPECTIVE EVALUATION ON EFFICACY AND FEASIBILITY OF R-CODOX-M/IVAC REGIMEN IN AGGRESSIVE DLBCL

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Background: Diffuse Large B Cell Lymphoma (DLBCL) is an heterogeneous group of diseases. The aggressive behavior can be predicted by clinical risk scores, immunohistochemistry and cytogenetic. Among DLBCL, double hit lym-
phomas (DHL) and double or triple-protein-expression lymphomas (DPLs, TPLs) display a worse outcome. R-CHOP, which is the frontline treatment for DLBCL, showed a poor outcome in high risk IPI patients and DHLs or DPLs. From January 2011 in our centre (IRCCS AOU San Martino Hospital–IST, Genoa, Italy) R-CODOX-M/IVAC regimen has been adopted as first line in patients with aggressive DLBCL, defined by at least one among these features: high tumour burden, DPLs, IPI score >3 or by the presence of at least 1 extranodal site.

Aims: Our aim was to define the efficacy and feasibility of this frontline strategy and eventually identify the subgroups of patients who may benefit from this approach.

Methods: We retrospectively analyzed 20 patients affected by aggressive DLBCL treated with R-CODOX-M/IVAC. R-CODOX-M consists of rituximab 375 mg/m² day 1, cyclophosphamide 800 mg/m² day 1, 200 mg day 2-5, doxorubicin 40 mg/m² day 1, vincristine 1.4 mg/m², and methotrexate 6700 mg/m². IVAC-R contains rituximab 375 mg/m², ifosfamide 1500 mg/m² day 1-5, etoposide 50 mg/m² day 1-5, cytarabine 2000 mg/m² bid day 1-2. 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 (55%), partial remission in 2 patients (10%). The overall response rate was 82%. Three patients (15%) were not assessable due to DPLs. OS after 6 cycles of chemotherapy was a twelve months was 88.9 and 64.8%, respectively, not significantly lower than non DPLs 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 78.6 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 hematological ones 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 tend to have a longer OS, the median OS was 33 months (9-not reached), while normal weight patients tended to have a longer OS, the median OS not reached. The worse survival among non-antracyclin regimen treated patients, had obese patients, median OS 26 months (9-not reached). Overweight females and men with normal weight patients were the best survivors and the survival was slightly better in females than in males (p=0.03). Obesity was associated with shorter survival among older patients with DLBCL treated with different chemotherapy regimens. The impact of gender on PFS and OS varied with BMI. The use of antracyclin did not influence the outcome of obese patients. This study suggests that BMI may predict survival in older patients with newly diagnosed DLBCL.

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 cell lymphoma. Immunochemotherapy followed by consolidation radiation is the current standard of treatment. However, the cycles of chemotherapy and the role of consolidation radiation are still under debate.

Aims: To review and analyze the treatment experience of newly diagnosed primary gastric diffuse large B cell lymphoma. We presented the treatment outcome of our institution.

Methods: We retrospectively reviewed medical records from Jan 2005 to Dec 2014 from our institution. 30 patients with primary gastric diffuse large B cell lymphoma were included. Clinical characteristics, treatment regimens, treatment response, treatment modulation, 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). RCHOP 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 remission (CR), 5-year survival for 4 cycles of chemotherapy was 88% vs 86% (p=0.42), respectively. For addition of IFRT in CR patients, 5-year survival for IFRT vs no IFRT were 83% vs 90% (p=0.93), respectively. Treatment-related mortality (TRM) was 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.

Summary/Conclusions: In our series, the 5-year survival was good. In patients who achieved CR, cycles of chemotherapy and consolidation radiation did not make significant difference to the survival. Prevention of early mortality may improve the outcome of this disease. Gastrointestinal bleeding in treatment is rare but with high mortality.

PB1735

IMMUNOHISTOCHEMISTRY BIOMARKERS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY

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Background: Diffuse Large B-Cell Lymphoma (DLBCL) is a heterogeneous group of lymphomas with variable clinical outcomes. The International Prognostic Index (IPI) is the most important tool to identify subgroups with different survival, however, certain biological markers seem to have a prognostic value relevant and independent of IPI.
Aims: To analyze the evolution of patients diagnosed with DLBCL and the expression of BCL2, BCL6, and MYC.

Methods: We conducted a retrospective study that included hospitalized patients with de novo CD20+ DLBCL, with expression of BCL2+, BCL6+, BCL2/BCL6, MYC/BCL2, MYC/BCL6 treated with regimens containing rituximab, from February 2012 to November 2016. Samples were analyzed by immunohistochemistry. Statistical analysis with the SPSS V17.0 program.

Results: We included 43 patients with a median age of 65 years (22-97), 59.5% male, 45.2% had IPI 0-2, 54.8% had IPI 3-5, 26.2% stage I-II, 73.8% stage III-IV, 61.9% had extranodal disease and 23.8% bulky disease. Ki-67 was elevated in all patients who did this evaluation (n=28). In 13 patients was identified BCL2/BCL6+ in 6, and 21 patients had co-expression of BCL2/BCL6, 1 patient had MYC/BCL2 and 1 had MYC/BCL6. The R-CHOP regimen was first line treatment in 92.8% of patients. The ORR was 82.5%, with 65% of CR, 15% PR and 17.5% PD. Of those patients who received second line treatment, 8 expressed BCL2/BCL6, 4 BCL2, 2 BCL6, 1 MYC/BCL2, and 1 MYC/BCL6. Of these patients without second line treatment 3 expressed BCL2/BCL6, 1 BCL2, and 1 MYC/BCL6. The average time to next treatment (TNT) was 5.2 months (0.5-19) for second line and 4.9 for third line. Mortality rate was 45.2%. With a median follow-up of 18.6 months (3-58.6), the overall survival was 24.6 months (3-62).

Summary/Conclusions: The identification of biomarkers by immunohistochemistry is a relatively inexpensive process, which, when well elaborated and interpreted, allows to find in a safe way, subgroups of patients at high risk, who benefit from more aggressive 1st line therapy and, whenever possible, from the Inclusion in clinical trials with new drugs.

PB1736
INVESTIGATION ON TREATMENT STRATEGY, PROGNOSTIC FACTORS, AND RISK FACTORS FOR EARLY DEATH IN ELDERLY TAIWANESE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Given that the population of elderly cancer patients, including those with diffuse large B-cell lymphoma(DLBCL), is increasing, the management of cancer in the elderly has emerged as an increasingly common problem.

Aims: This study aimed to investigate the treatment strategy, prognostic factors, and risk factors of early death in elderly patients (age ≥65 years) with DLBCL in the rituximab era.

Methods: Elderly patients diagnosed with DLBCL between 2008 and 2014 were enrolled for analysis.

Results: There were 145 elderly patients with DLBCL diagnosed between 2008 and 2014. After excluding patients with primary central nervous system DLBCL (n=9) and incomplete data (n=3), a total of 133 patients (64 male and 69 female) with a median age of 74 years (range 65 to 94 years) were enrolled in the present study. Patients at a younger age and with better performance status were more likely to receive intensive frontline treatment. The median progression-free survival (PFS) and overall survival were 15 and 21 months, respectively. Anthracycline-containing chemotherapy achieved a higher remission rate and showed a trend toward better overall survival at the expense of a higher risk of severe neutropenia. Multivariate analysis revealed that very old age (≥81 years), a high-risk age-adjusted international prognostic index (aaIPI) score, and bone marrow involvement were associated with poorer PFS and overall survival. Progression of lymphoma was the major cause of death in the study population. In addition, approximately 25% of patients died within 120 days of their diagnosis. The risk factors for early mortality included very old age, a high-risk aaIPI score, and bone marrow involvement. The appearance of symptoms or signs of tumor lysis syndrome at diagnosis was associated with a trend toward early death.

Summary/Conclusions: Treatment of elderly patients with DLBCL requires a specialized and comprehensive evaluation 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 EPITHELIOTROPIC INTESTINAL T-CELL LYMPHOMA: EXPERIENCE FROM AN ASIAN CANCER CENTER
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Background: Monomorphic epitheliotropic 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, NOS. Four patients were diagnosed (probable) monomorphic epitheliotropic 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 received chemotherapy followed by chemotherapy as maintenance (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.
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 validate 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.

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 chemotherapy 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.

Figure 1.
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: 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.

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.

Results:
Total of 505 lymphoma patients was included. Median age was 69 (range 20-99). 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 (3.2%). There was no difference of distribution between patients with and without sMPMts regarding age, grade of lymphoma, and disease risk. The overall survival (OS) and disease-free survival (DFS) were not significantly different between the two groups (with sMPMts: 53% and 47% vs without sMPMts: 77% and 61% at 3 years, P=0.20 and P=0.31). Cumulative incidence of lymphoma relapse was similar between the two groups (with sMPMts 29% vs without sMPMts 27% at 3 years, P=0.28). In multivariate analyses, age (75 years<) and disease risk (high) were identified significant risk factors for OS, and age was an only significant risk factor for DFS. Existence of sMPMts was not a significant risk factor for either OS or DFS (OS: HR 1.29, 95%CI 0.52-3.20, P=0.58; DFS: HR 1.06, 95%CI 0.49-2.27, P=0.88). Among 16 patients with sMPMts, half of the patients had high-risk lymphoma, and half of the solid tumors were gastric cancer. Treatment was initiated for the disease which was diagnosed earlier in all patients except one. Interval from diagnosis to the first treatment was significantly shorter in patients whose lymphoma was treated earlier (median 11 days vs 38.5 days, P<0.004). OS was not significantly different according to the sequencing of treatment (lymphoma earlier: 59% vs Solid tumor earlier: 40% at 3 years, P=0.84). In 8 of 10 patients whose lymphoma was treated earlier, treatment of lymphoma was interrupted for the treatment of sMPMts. Interruption of treatment had no significant effect on OS (interuption+: 60% vs interruption-: 50% at 3 years, P=0.13).

Summary/Conclusions: Existence of sMPMts was not a significant risk factor for newly diagnosed lymphoma patients. It is important to provide adequate treatment for both lymphoma and solid tumor at physician’s discretion.
Bleeding disorders (congenital and acquired)

PB1743

GLOBAL HEMOSTATIC ASSAY AT DIFFERENT TARGET ACTIVITY OF FACTOR VIII AND FACTOR IX

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Background: Based on reports addressing hemophilia B patients bleed less common and less intensively than hemophilia A, it has been expected that the hemostatic level of factor IX (FIX) activity can be lowered 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 50% in hemophilia A and 40% in hemophilia B which is given by Korean health insurance guideline. All patients were in non-bleeding state and kept the wash-out period of 3 days for hemophilia A and 5 days of hemophilia B. Before and on 15 minutes after injections, we conducted one-stage factor assay, thrombin generation assay (TGA), thromboelastography (TEG) and clot-wave form analysis (CWA).

Results: Median ages of hemophilia A and hemophilia B patients were 28 and 33 years old. Baseline FVIII:C and FIX:C were 0.6% and 1.8% and they rose after injection rose to 70.8% and 49.8%. The dosage of FVIII concentrates and recombinant FIX concentrates were 28.4 IU/kg and 50.7 IU/kg. In vivo recovery (IVR) in A 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~+2). 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 FVIII and FIX are normal, less amount of FIX is insufficient to normalize hemostatic parameters in comparison with FVIII.

PB1744

THE RATE OF SUCCESSFUL IMMUNOTOLERANCE INDUCTION IN HAEMOPHILIA A BOYS TREATED WITH OCTOCOG ALFA - THE EXPERIENCE OF POLISH PAEDIATIC HAEMOPHILIA CARE CENTRES

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2Medical Faculty, University of Novi Sad, 3Clinic for gynecology and obstetrician, 4Clinic for abdominal and endocrine surgery, 5Department for thrombosis, hemostasis and hematology diagnostic, 6Center for Medical Genetics, Institute for Children and Youth Health Care of Vojvodina, Novi Sad, Serbia

Background: Niemann Pick Disease type A and B is a rare autosomal recessive disorder caused by sphingomyelinases deficiency resulting in sphingomyelin accumulation in macrophages of various organs. Type B usually patients survive in adulthood. Usually, they have hepatosplenomegaly, thrombocytopenia, and dyslipidemia and liver and lung function are impaired, and they have a 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 Niemann Pick disease type B. She had marked splenomegaly, mild thrombocytopenia and partial respiratory insufficiency. Previously, she had two artificial abortions 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. Laboratory controls were done periodically. Ultrasound 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 cholesterolemia was present. We assumed that platelets dysfunction could exist, therefore before gestation 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). Ultrasound examination of abdomen and portal vein system revealed liver diameter 17cm, cranio-caudal diameter of spleen 22cm, portal vein had not been seen. There were no signs of portal thrombosis in portal branches. Aminoacid test was done without complications and there was no need for platelet substitution. Normal male kariotype was found. We prepare her for planned caesarian section with platelet concentrates. She was given corticosteroids for lung maturation. In 35+5 gestational week she was operated. Before surgery platelet count was 87x109/L, she was given seven concentrates of platelets (1 per 10 kg body weight) before and seven during procedure. 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. We decided not to make splenectomy or partial resection because there were no significant differences in spleen measurements before and during the pregnancy, and there was no sign of spleen trauma. Also, in literature we found data about worsening lung function after this procedure caused by more sphingomyelin accumulation in pulmonal tissue. Published data and findings of abnormal platelet function in our patient and experience with previous abdominal 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 findings 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 assessment, platelet transfusion, splenectomy, and

Patient

Table 1. Characteristic of patients.

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OD, on demand; P, prophylaxis; CVA, central venous access; N, no; V, yes; mth, month.
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: %0. Two years later underwent a planned tooth operation under the coverage of prothrombin complex concentrate (PCC) (Table 1). Three years after the tooth extraction he underwent an intraocular lens operation under PCC prophylaxis. No complication was observed while on PCC treatment.

Table 1.

<table>
<thead>
<tr>
<th>weight : 70 kg</th>
<th>PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation day</td>
<td>750 unit</td>
</tr>
<tr>
<td>2nd day</td>
<td>500 unit</td>
</tr>
<tr>
<td>4th day</td>
<td>500 unit</td>
</tr>
<tr>
<td>6th day</td>
<td>250 unit</td>
</tr>
</tbody>
</table>

Our second patient is a woman who was diagnosed at the age of 3 because of recurring gum bleeding. She has been treated with FFP replacement throughout her childhood and adolescence due to recurring nose and soft tissue bleeds as well as menorrhagia. She was first referred to our hospital at the age of 42 due to soft tissue bleeding. Given the lack of health insurance she mainly received FFP and tranexamic acid tablets during most of her bleeding attacks. However, PCC of 1000 unit for two days had to be used for her excessive vaginal bleeding irrespective to FFP. Her number of annual bleeding is 15-20 times in a year and most of them are gum bleeding and rarely vaginal bleeding. Third and 4th patients were referred to our centre because of prolonged the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) and received the diagnosis of FX deficiency.

Summary/Conclusions: Bleeding phenotype differs in a wide range in patients with congenital FX deficiency. Secondary causes including amyloidosis should be excluded especially in patients receiving diagnosis at advanced ages. Usually the factor level does not correspond to the severity of the bleeding phenotype. Therefore bleeding pattern of the patients with FX deficiency should be carefully observed and considered while planning a prophylactic treatment with PCCs to prevent the risk for thrombosis and unnecessary utilisation of PCCs. FFP and PCCs replacement continue to be the source for FX in bleeding patients or in individuals requiring prophylaxis. Recently, a FX concentrate has entered the market in the USA and the European Community.

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 (≥4). Those with high bleeding scores, three were obligate carriers and four were probable carriers. The mean factor activity of 12 obligate and 18 probable carriers were 78.9% (20.8%>189%). Factor activities of the three obligate carriers with high bleeding scores were 77%, 80% and 98%, respectively. Factor activities of the three probable carriers with high bleeding scores were 58.8%, 69.3% and 112%, respectively. The median bleeding scores of four probable and one obligate carriers with low factor activity (<60%) were 2.8 (1-4).

Summary/Conclusions: Measurement of factor activity seems to be insufficient to detect hemophilia carriers. ISTH/SSC-BAT may help to determine the carriers. However, a larger study is needed to understand the diagnostic value of the BAT.

PB1749

FETAL INTRACRANIAL HEMORRHAGE AS A PRESENTING FEATURE OF SEVERE CONGENITAL FACTOR VII DEFICIENCY: THE NEED FOR EARLY PROPHYLAXIS
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Background: Congenital factor VII (FVII) deficiency is a rare autosomal reces-
sive bleeding disorder, with an estimated prevalence of 1:300,000. Compared to western countries, rare bleeding disorders (RBDs) are relatively commoner in Oman, owing to high rate of consanguinous 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.

**Summary/Conclusions:** Children with RBDs constitute more than one tenth of cases of hereditary coagulation factor deficiencies in our center. They have some unique features in terms of severity, clinical profile and the need for prophylaxis early in life. We recommend establishing a national/regional registry of RBDs to identify the magnitude and the peculiar genotype-phenotype correlations of such rare, yet significant disorders.

**PB1750**

**THE ASSOCIATION OF BLOOD TYPE WITH THE NEED FOR TRANSFUSIONS IN PATIENTS WITH VENTRICULAR ASSIST DEVICES**

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**Background:** Patients who have implantation of continuous flow ventricular assist devices (VAD) as a bridge to heart transplantation are subjected to complications secondary to pump support. The use of antiplatelets either alone or in combination with anticoagulation is necessary to avoid clot formation and pump thrombosis. However, a proportion of patients reveal an increasing risk of bleeding episodes. A possible reason of this situation could be that high shear forces lead to devastation of high molecular weight von Willebrand factor (vWF) making it functionally inactive and resulting in acquired von Willebrand disease (vWD). People with blood type O have lower baseline vWF levels and this abnormality could exacerbate the bleeding risk of patients with blood type O and 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. 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 lymphocyte frequently had STAT3 mutations (Oie ZY et al. J Hematol & Oncol 2013, Ishida F et al. Cancer sci 2014). Molecular or flow-cytometric analysis is useful for detecting a small amount of abnormal lymphocytes.

Aims: We conducted this study to determine the clinical characteristics and STAT3 mutations of patients with acquired PRCA on dialysis with lymphoproliferative diseases.

Methods: In our hospital, 4 patients were diagnosed as having acquired PRCA on dialysis with lymphoproliferative diseases after 2005. Patients were retrospectively studied for presenting feature, laboratory data, and clinical course. Surface markers of lymphocytes were examined by flow cytometric analysis, and T-cell receptor (TCR) rearrangements were examined by Southern blot analysis. Mononuclear cells were separated after obtaining written informed consent. STAT3 (Y640F and D661Y) mutations were examined by allele-specific PCR. Current study was conducted within the guidelines and with the approval of the institutional ethical committee.

Results: In spite of adequate administration of erythropoietin colony-stimulating factor, all 4 patients required blood transfusion due to erythropoietin-refractory anemia. 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 62% of lymphocytes were useful for diagnosis of acquired PRCA associated with lymphoproliferative diseases. Further accumulations of patients were required for understanding the pathogenesis of lymphoproliferative diseases causing acquired PRCA on dialysis.

PB1753
REACTIVATION OF HEPATITIS B VIRUS INFECTION IN APLASTIC ANEMIA PATIENTS
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22nd Congress of the European Hematology Association

Background: There is litter 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 patients receiving CsA and/or ATG.

Methods: We analyzed the clinical data of 60 AA patients with HBV infection during the follow-up period. The follow-up period was from the start of dialysis therapy to the diagnosis of PRCA of patients (range, 5-19 years). Of the 4 patients, only one patient (Case 3) had the mutations of the STAT3 gene (Y640F). This patient first received cyclophosphamide but he did not respond to the therapy. He subsequently received cyclosporine (CyA). The other three patients received CyA as an initial therapy, and it was effective in all 4 patients. Median follow-up was 7 years from diagnosis, and two patients died during follow-up period. One patient (Case 4) died of cardiac failure 7 years from the diagnosis. Another patient (Case 2) developed diffuse large B-cell lymphoma 5 years after the administration of CyA. He was treated with R-CHOP chemotherapy and complete remission (CR) was achieved. Although he had been in CR, he died of refractory pancytopenia with infection, 2 years after the lymphoma onset. The other two patients are still alive without blood transfusion for 6 and 7 years.

Summary/Conclusions: A proportion of erythropoietin-refractory anemia patients on dialysis have acquired PRCA associated with lymphoproliferative disease. Flow cytometry analysis of flow cytometry and TCR C beta1 and gamma rearrangements of lymphocytes are useful for diagnosis of acquired PRCA associated with lymphoproliferative diseases. Further accumulations of patients were required for understanding the pathogenesis of lymphoproliferative diseases causing acquired PRCA on dialysis.

Summary/Conclusions: The patients with relapse or refractory PRCA, there were no satisfactory treatment measures if CsA and/or CsA were not be administered or un-effective. It was still needed to explore a more effective therapy for them.

PB1754
MULTICENTER RESULTS OF SCHWACHMAN-DIAMOND SYNDROME PATIENTS
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2Cerrahpasa University, Department of Pediatric Hematology, Istanbul, Turkey
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5Biyokimya ve Patoloji, Ankara, Turkey
6Dokuz Eylül Üniversitesi, Medical Faculty, İzmir, Turkey
7İstanbul Saglik Bilimleri University, Department of Pediatric Hematology, Istanbul, Turkey
8Hacettepe University, Department of Medical Genetics, Ankara, Turkey
9Hacettepe University, Division Of Pediatric Hematology, Ankara, Turkey

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 were selected from 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 slight bony abnormalities. Failure to thrive character was found in 75% 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 pancytopenia might be the hematological presentational findings of SDS.

PB1755
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA AND APLASTIC ANEMIA—DATA FROM THE SPANISH PNH REGISTRY
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Background: Aplastic anemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH) are included, together with other pathologies, within the bone marrow failure syndromes (BMFS). In the present time, these clinical entities cannot be understood as independent pathologies, due to the extremely frequent evolution among them and with other BMFS, along with the development of new clones in the context of haematopoietic stem cell’s kinetics.

Aims: The aims of this study were analyzing and comparing the behaviour of patients who suffered from PNH with pancytopenia with respect to that of patients who were initially diagnosed of AA and who later developed a PNH clone.

Methods: A clinical form was elaborated and distributed among the investigators of the PNH Spanish Registry. Clinical, laboratory and treatment data of the patient were asked. Soon after, a descriptive analysis of the data was performed.

Results: 34 patients were recruited and analyzed (12 women and 22 men). Their age interval ranged from 2 to 87 years, and all of the patients suffered from either PNH with pancytopenia and/or AA with a developing PNH clone. The average age of the patients at the time of initial diagnosis was 28.5 years old (4-72y). The failure to thrive was PNH with pancytopenia in 50% of the patients, while in the genetic verified PNH, the clinical evolution should be understood as independent pathologies, due to the extremely frequent evolution among them and with other BMFS, along with the development of new clones in the context of haematopoietic stem cell’s kinetics.

Summary/Conclusions: We have analyzed and compared, for the first time, the PNH patients with severe AA with another PNH patients. In the present time, these clinical entities cannot be understood as independent pathologies, due to the extremely frequent evolution among them and with other BMFS, along with the development of new clones in the context of haematopoietic stem cell’s kinetics.

PB1756
AUTOIMMUNE CYTOPENIAS IN PRIMARY IMMUNODEFICIENCY DISEASES: SINGLE CENTER EXPERIENCE
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Aims: Primary immunodeficiency diseases (PIDs) are associated with hematologic complications such autoimmune hemolytic anemia (AIHA) and thrombocytopenia (ITP). The most common autoimmune cytopenia is ITP. Although ITP is observed in 7.6% of patients with PID, AIHA is seen at 4.8%. Also, we aimed to present the patients who had autoimmune cytopenias and PID.

Methods: Fifty six PID patients who were followed at the Pediatric Immunology Department of Erciyes University Medical Faculty (they were analyzed genetically) were evaluated retrospectively. Autoimmune cytopenias such as ITP and AIHA were detected in 9 (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 OCS 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 paradoxal situation between PID and autoimmunity. The reduction of central and peripheral tolerance is held responsible for autoimmunity in PID.

Summary/Conclusions: As a conclusion, we wanted to point out autoimmune cytopenias in patients with PID and the requirement of multidisciplinary approach for treatment.

PB1757
HEAVY METAL LEVELS IN PATIENTS WITH FANCONI APLASTIC ANEMIA
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Background: Fanconi aplastic anemia (FAA) is a rare, autosomal recessively inherited bone marrow failure disorder. Various congenital anomalies may accompany disease and various complications including malignancy and endocrinopathies may develop during the course.

Aims: Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA. Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA.

Methods: Study was performed between July 2015 and April 2016 among patients with FAA and the results were compared with age and gender matched patients with non-neoplastic hematological diseases. Plasma cobalt (Co), chromium (Cr), zinc (Zn), nickel (Ni), cadmi- um (Cd), lead (Pb) and selenium (Se) levels were measured in patients with FAA.

Results: Total of 17 patients with FAA were included in the study. Median age was 9 years (1-30), female to male ratio was 8/9. One patient had undergone stem cell transplantation, four patients were transfusion dependent. When we compared patients with FAA and age/sex matched healthy group (16 volunteers), Cr and Cu levels were higher and Se level was lower in FAA group significantly (Table 1). However, all patients had chromium level within normal range, two patients with FAA and two volunteers had copper levels higher than the normal ranges (Table 2).

Table 1. Heavy metal levels in patients and control group.

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>FAA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium (mg/L)</td>
<td>0.3 (0.3 – 0.5)</td>
<td>0.25 (0.2 – 0.35)</td>
</tr>
<tr>
<td>Cadmium (mg/L)</td>
<td>0.05 (0.03 – 0.07)</td>
<td>0.03 (0.02 – 0.04)</td>
</tr>
<tr>
<td>Copper (mg/L)</td>
<td>112 (105 – 119)</td>
<td>108 (100 – 110)</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>187 (179 – 192)</td>
<td>190 (180 – 200)</td>
</tr>
<tr>
<td>Nickel (mg/L)</td>
<td>44.4 (36.8 – 50.6)</td>
<td>41.2 (35.0 – 47.0)</td>
</tr>
<tr>
<td>Selenium (mg/L)</td>
<td>77.1 (47.3 – 133.0)</td>
<td>68.9 (46.0 – 98.0)</td>
</tr>
</tbody>
</table>

FAA: Fanconi aplastic anemia.
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 general geriatric population and can either hamper diagnostic evaluation or increase the complexity of PNH patient care.

Aims: To identify geriatric-age PNH in Spanish PNH registry. To study the clinical characteristics at diagnosis and evolution of geriatric-age PNH and compare them to non-geriatric PNH population. To analyse the impact of both age and comorbidity in the PNH setting. To evaluate the use of eculizumab in geriatric age patients.

Methods: In a multicentric retrospective study, Cumulative Illness Rating Scale for Geriatrics (CIRS-G) and clinical and biological variables have been collected from a Spanish PNH Group patient cohort. Statistical analysis was performed using GraphPad Prism v6 (La Jolla, CA).

Results: 44 patients from 11 centres in Spain have been included up to date. 8 patients (17.8%) were diagnosed in geriatric age (equal or older than 65 years) (Age range for the complete cohort: 17-83 years) and 9 patients presented with high comorbidity, arbitrary defined as CIRS-G score >10. (Range for the geriatric cohort: 3-13) Age and comorbidity were poorly correlated (p=0.0187, R-square 0.15). No differences in clinical presentation (Classic, PNH in the setting of another bone marrow failure syndrome or Subclinical PNH or high disease activity) when stratifying by age or comorbidity were observed. 4 patients had a concomitant myeloid clonal disorder (3 myelodysplastic syndrome and 1 myeloproliferative neoplasia), 3 of them (75%) in geriatric age. Median follow up was 7.2 years. Both age equal or older than 65 years and CIRS-G >10 were associated to poorer overall survival (HR: 0.0134 and 0.045 & p=0.0015 and 0.103 respectively). Regarding PNH with high disease activity, 18 patients were identified, 4 of them in geriatric age. In 2 of them (50%), Eculizumab was used, which contrasts with eculizumab use in younger patients (78.6% in the same indication) Regarding comorbidity impact on eculizumab therapy outcome, 2 patients had CIRS-G score >10 and had similar overall survival as patients with lower comorbidity in this cohort.

Summary/Conclusions: Age and comorbidity are associated with poorer overall survival in PNH. Older age and comorbidity may not preclude the use of effective treatment in PNH patients, including those with high disease activity. Prospective evaluation of comorbidity in PNH patients, regardless of age is warranted.
Chronic lymphocytic leukemia and related disorders - Biology

PB1760

LDH AS PREDICTIVE PARAMETER IN TREATMENT-NAÏVE PATIENTS WITH TRISOMY 12 CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Patients affected by chronic lymphocytic leukemia (CLL) that have trisomy 12 (+12) on FISH analysis have unique clinical and biological features. In a prior analysis (Autore F, ASH 2016) of 487 patients with +12 compared to 816 patients with negative FISH, patients with +12 had a significantly higher prevalence of elevated LDH, β-2-microglobulin, ZAP70 positivity, CD38 positivity, CD49d positivity and unmutated IGHV as compared to patients with negative FISH. They also showed shorter progression free survival (PFS), treatment free survival (TFS) and overall survival (OS)

Methods: This study included 487 treatment-naïve patients with +12 CLL from 16 academic centres, diagnosed between January 2000 and July 2016. A cohort of 250 patients with +12 CLL followed at a single US institution was used as external validation. Data were summarized as medians and 25th and 75th percentiles. Chi-square test or Fisher’s exact test were used to compare categorical variables, while Wilcoxon-Mann-Whitney-Test was applied for continuous variables. The survival analysis was based on the Kaplan-Meier method and the log-rank test was used to compare survival curves. A Cox model was used for multivariate analysis of the impact of different factors on survival. P values lower than 0.05 were considered statistically significant (STATA 12.0) and reported as two-sided. We analysed also CLL-specific survival considering events deaths due to the haematological disease.

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 unmaturated IGHV remained significantly correlated with shorter PFS, TFS, OS and CLL-specific survival, higher Rai stage with shorter PFS and elevated β-2-microglobulin with shorter OS. Considering interestingly the association of a simple and new laboratory parameter such as LDH to the outcomes, confirmed on multivariate analyses for PFS (hazard ratio [HR] 1.55, 95% confidence interval [CI] 1.2-2.2, p<0.002), TFS (HR 1.65, 95% CI 1.2-2.2, p=0.034) and CLL-specific survival (HR 3.86, 95% CI 2.0-7.5; p<0.001), we divided our +12 CLL cohort according to LDH levels available at diagnosis: 103 patients showed LDH levels above the normal limit and 184 within normal range. Patients with high LDH levels showed shorter PFS (30 months vs 65 months, p<0.001; Figure 1A), TFS (33 months vs 69 months, p<0.001; Figure 1B), OS (131 months vs 181 months, p<0.001; Figure 1C) and CLL-specific survival with a rate of attributable mortality of 29% vs 11% (p<0.001). In the validation cohort, 104 patients had high LDH levels and 145 patients had normal LDH levels; factors significantly associated with PFS and TFS on univariate analysis were LDH, β-2-microglobulin, Rai stage and ZAP70; LDH, β-2-microglobulin and age associated with OS. On multivariate analysis high LDH was the sole parameter significantly associated with all shorter outcomes, along with elevated β-2-microglobulin, which associated with worse outcome.

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 CONFRONTS PROGNOSTIC INFORMATION IN CLL PATIENTS

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Background: Genomic aberrations detected by FISH have become one of the most important and widely used prognostic factor for chronic lymphocytic leukemia (CLL) patients. In addition several publications have described that patients with a higher percentage of abnormal nuclei have a worse outcome.

Aims: To analyze the effect of the percentage of abnormal nuclei detected by FISH (13q deletion (13q-), 11q deletion (11q-), 17p deletion (17p-) and trisomy 12 (+12)) in overall survival (OS) and time to first treatment (TTFT).

Methods: We studied a non-selected cohort of 650 consecutive CLL cases from a local database with a median follow up time of 50 months (0-346). The cut-off point for the percentage of abnormal nuclei for each alteration was detected by dividing the variable into deciles, and selecting the most efficient cut-point, and based on previous publications.

Results: FISH detected aberrations in 85% of the cases (442/505). The most frequent abnormality was 13q-, observed in 302 patients (47%), but as a sole alteration in 212 cases, followed by +12 (106 patients, 16%), 11q- (83 patients, 13%), and 17p- (33 patients, 5%). As expected, the group of patients with 13q- as a sole abnormality was the one with the better OS (195 months) followed by the group of patients with normal FISH (160 months), +12 (124 months), 11q- (56 months) and 17p- (46 months), consistent with the 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 observed.

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.

Table 1.

![Table 1](https://example.com/table1.png)
Background: Chronic lymphocytic leukemia (CLL) pathogenetic mechanisms have not been fully elucidated yet. However, genetic and epigenetic alterations seem to be involved in the pathogenesis and extensive clinical heterogeneity of the disease. DNA methylation in CpG sites of a gene promoter, which may affect the chromatin structure as well as gene transcriptional activity, is a crucial epigenetic modification in CLL. RAD21 gene is involved in DNA repair and its encoded product acts as basic subunit of the Cohesin protein complex that regulates the cohesion and proper separation of sister chromatids during mitosis or meiosis.

Aims: We investigated the methylation status of RAD21 gene promoter and its possible implication in CLL pathogenesis and the formation of CLL cytogenetic aberrations.

Methods: The study included 105 CLL patients and 17 healthy donors (controls). Total genomic DNA extraction was performed from bone marrow or peripheral blood samples of all patients and controls. Methylation analysis of RAD21 gene promoter was carried out using the new technology of MethylScreen™ in the FX96Biorad 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 enzymes. Methylation analysis was performed on unmethylated and methylated with CpG-oligonucleotide DSP-30 bone marrow cells of CLL patients. FISH analysis was carried out using the commercial CLL SET probes for detection of the most common abnormalities of the disease including deletions of 17p13 (TP53), 11q22.3 (ATM) and 13q14.3 (BRCA1/3) 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 or/and FISH analysis. Methylation study was successful in all healthy donors and in 101 out of 105 CLL patients. All healthy donors had non-methylated RAD21 gene promoter. Contrary, 25.74% (26/101) of CLL patients carried >10% cells with methylated CpG islands in RAD21 promoter, which was significantly increased compared to controls (p=0.039, \( \chi^2=4.25, df=1 \)). RAD21 methylated cell fraction varied among patients. More specifically, 8.9% of patients (10/101) showed 11-50% methylation rate, 10.89% (11/101), 25.74% (26/101) and 4.95% (5/101) 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 t(11/16) (with abn(14q6)), in 33.33% (4/12) with abn(6), in 31.25% (5/16) with -17del(17p), in 27.78% (7/26) with trisomy 13, in 25.81% (8/31) with del(13q), in 20% (2/10) with del(6q) and in 12.5% (2/16) with del(11q). Based on karyotypic complexity, RAD21 promoter was methylated in 18.18% (4/22) of patients with a single chromosome aberration, 26.09% (6/23) with two chromosome aberrations and 25.71% (9/35) of patients with complex karyotype (≥3 aberrations).

Summary/Conclusions: Methylation of RAD21 gene promoter, which leads to transcriptional inactivation and consequent inhibition of RAD21 expression, seems to be implicated in CLL pathogenesis and the formation of specific chromosome aberrations. Clarification of the epigenetic landscape of CLL may help in the design of new targeted therapeutic agents.

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 cellular antioxidant defenses. The number factor erythroid 2-like gene, 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 in the pathogenesis of such diseases is not fully elucidated.

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-AMPS-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 survival curves by estimating the progression free survival (PFS) and overall survival (OS).

Results: The results showed that individuals with the GG genotype (NFE2L2) are at higher risk of developing CLL (Odds ratio (OR): 2.032; 95% confidence interval (CI): 1.234-3.57; p=0.004). In the genotypic profile (GP) GG / GC (NFE2L2 / KEAP1) is a risk factor (OR: 2.180; 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 1.030-3.303, p=0.037; KEAP1 CC: OR 1.2, 95% CI 1.041-3.777, 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 / GC patients have a lower mean survival (198.0±13.6 months) than patients with other genotypes (198±13.4 months; p=0.023)].

Summary/Conclusions: This study suggest that genetic polymorphisms in NFE2L2 and KEAP1 genes might be risk factors for CLL development and may constitute novel genetic markers for therapy response (namely regimes with rituximab and fludarabine) as well as prognostic markers, by influencing overall survival and progression free survival in CLL patients. The authors declare no conflicts of interest.
Results: An increased number of CAs, including chromatid breaks and dicentrics, in CLL patients (6.59±5.3%) compared to controls (0.25±0.04%) (p=0.021) was observed. A tendency to increased CA frequency in cases with abnormal (8.18±4.1%) 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 (5.45±3.5%). By MN analysis, an increased frequency in CLL patients (2.81±1.5%) compared to controls (0.67±3.0%) (p=0.0001) was found. Patients with +12 presented the highest percentage of MN compared to the other two groups (+13, 11q22), 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±2.4%, respectively) and UM-CLL (6.2±5.8% and 2.7±3.1%, 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 mechanisms of DNA damage.

PB1765

B CELLS RESISTANT TO CD20 MONOClonAL ANTIBODIES DISPLAY SPECIFIC ALTERATIONS IN GENE EXPRESSION PROFILE

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Background: CD20 monoclonal antibodies (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 chronic exposure to gradually increasing doses of monoclonal antibodies, we have generated cell lines that are resistant to additional treatment with mAb. Notably, these cells are resistant also to any other of the available anti-CD20 antibodies even at very high concentrations as shown by dose-response experiments. This resistance is sustained for long period and maintained even upon marked reduction of antibody concentrations. We could also show that these cells with regulated CD20 protein from the cell surface and that this effect was not just due to its internalization. Instead, we detected a defect in CD20 transcription as measured by quantitative real-time PCR. Flow cytometry analysis of other surface markers showed a strong upregulation of CD55 and CD59, known inhibitors of complement activation. The combination of CD20 loss together with the increase of CD55 and CD59 is responsible for the complete resistance to the mAbs. We have then analyzed changes in overall gene expressions by performing RNA sequencing and quantitative real-time PCR. We have identified several interesting genes whose expression was altered in our resistant cells when compared to untreated cells. Among the most interesting hits was a strong downregulation of the transcription factor NFκB, which was expressed more than 10-fold lower in the rituximab or ofatumumab resistant cells. We could confirm this result in multiple independent experiments. We have postulated that anti-CD20-triggered signaling results in the inactivation of NFκB, thus blocking the block in TNFα transcription. To test this hypothesis, we have treated the cells with phosphoester PMA, which nonspecifically activates NFκB. Indeed, cells treated with PMA managed to rapidly upregulate CD20 on their cell surface.

Summary/Conclusions: In summary, CD20 triggering by therapeutic mAbs initiates complex intracellular changes that result in downmodulation of CD20 expression. Further analysis of detailed intracellular mechanisms regulating CD20 is warranted in order to propose novel interrogation nodes that might modulate CD20 surface density and thereby enhance the therapeutic potential of CD20 monoclonal antibodies.

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 (bCLL). Comprehensive profiling of chemokine receptors in CLL and its subgroups according to clinical course and cytogenetic aberrations 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 (WagnerGen 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-CR6, CCR8, CXCR4) were down-regulated in CLL. The expression of other receptors 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 immunochemotherapy, which is the aim of the present study.

Methods: Residual serum concentrations of rituximab were determined by an enzyme-linked immunosorbent assay (ELISA) for 35 CLL patients before each infusion, administrated every 28 days at T0, T1, T2, T3, T4, T5. Response and relapse criteria were evaluated according to the International Workshop on Chronic Lymphocytic Leukemia guidelines.

Results: Patients were assigned to two groups related to time to relapse. The first group (n=7), had an early relapse in less than 3 years, the second group (n=28) relapsed more than 3 years. 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 evaluated phases. Finally, statistically relapse criteria were met for rituximab serum concentration (AUC<sub>total</sub>=1.28±1.01 mgL<sup>-1</sup> day, AUC<sub>residual</sub>=2.79±1.93 mgL<sup>-1</sup> day, p=0.02). Additionally, the residual rituximab serum concentration between T2 and T5, superior at 70µg/ml, is associated with a longer 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, IDELALISIB OR VENETOLCLAX 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 idelisib, and the BCL-2 inhibitor, venetoclax.

Methods: The CLL cell line MEC-1 was treated with 0.3–10 μM ibrutinib, idelisib 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 antiproiferative activity or cytotoxic effects of the TKis, ibrutinib and idelisib, and the BCL-2 inhibitor, venetoclax.

Results: Ibrutinib and idelisib induced only moderate direct cytotoxicity on MEC-1 target cells but had strong antiproliferative effects. In contrast, venetoclax induced strong cytotoxicity on MEC-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 additional treatment with MOR208, idelisib 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, idelisib or venetoclax in vitro. These promising data provide a strong rationale for combination of MOR208 with these agents in future clinical trials.

PB1769

LYMPHOCYTE EXHAUSTION AND THE NATURAL HISTORY OF CHRONIC LYMPHOCYTIC LEUKEMIA – FRIENDS OR FOES?

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Background: Chronic lymphocytic leukemia (CLL) is a disease characterized by the appearance of morphologically mature monoclonal lymphocytes B with CD19+CD20+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 involved in lymphocyte exhaustion in the natural history of CLL. CLL is a clinically and biologically heterogeneous disease. As a consequence, novel biological and cytogenetic features have become increasingly important in predicting prognosis at the time of diagnosis and the research for molecules involved in apoptosis resistance and increased survival of neoplastic B cells is still ongoing.

Aims: We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in Chronic Lymphocytic Leukemia (CLL) B cells. Considering the crucial role of HSP70 in cancer, we aimed at characterizing this protein and its major 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 leukemia and normal B cells. HSP70 and HSF1 protein levels were correlated to IGHV mutational status and ZAP70 protein expression in CLL patients. HSP70 and HSF1 levels were also analyzed in neoplastic cells obtained from patients undergoing ibrutinib based regimen by WB analysis. Moreover, HSP70 and HSF1 localization was analyzed by subcellular protein fractionation followed by WB analysis. The effects of HSP70 and HSF1 inhibition by Zafirlukast and Fisetin were evaluated by Annexin V/Propidium Iodide flow cytometry test and WB analysis of PARP cleavage.

Results: We demonstrated that HSP70 and 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 underline 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 at steady state, suggesting a constitutive activation of these proteins in CLL. Although HSP70 has been extensively linked to cancer, little progress has 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.

PB1770

HSP70 AND HSF1 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 neoplasic disorder characterized by the accumulation of clonal B cells in peripheral blood, bone marrow and lymphoid tissues. CLL is a clinically and biologically heterogeneous disease. As a consequence, novel biological and cytogenetic features have become increasingly important in predicting prognosis at the time of diagnosis and the research for molecules involved in apoptosis resistance and increased survival of neoplastic B cells is still ongoing.

Aims: We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in Chronic Lymphocytic Leukemia (CLL) B cells. Considering the crucial role of HSP70 in cancer, we aimed at characterizing this protein and its major 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 leukemia and normal B cells. HSP70 and HSF1 protein levels were correlated to IGHV mutational status and ZAP70 protein expression in CLL patients. HSP70 and HSF1 levels were also analyzed in neoplastic cells obtained from patients undergoing ibrutinib based regimen by WB analysis. Moreover, HSP70 and HSF1 localization was analyzed by subcellular protein fractionation followed by WB analysis. The effects of HSP70 and HSF1 inhibition by Zafirlukast and Fisetin were evaluated by Annexin V/Propidium Iodide flow cytometry test and WB analysis of PARP cleavage.

Results: We demonstrated that HSP70 and 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 underline 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 at steady state, suggesting a constitutive activation of these proteins in CLL. Although HSP70 has been extensively linked to cancer, little progress has 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.

PB1771

OVEREXPRESSION OF GENE FOR HUMAN CONCENTRATIVE NUCLEOSIDE TRANSPORTER 3 IS A PREDICTOR OF RESISTANCE TO FLUDARABIN-BASED CHEMOTHERAPY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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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 q-PCR methodology, using TaqMan chemistry and ABI as endogenous control gene. Quantification of target gene expression was made by comparative ΔΔCT method using HLA-BL cell line as the calibrator. All analyses were done prior to any treatment.

Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with fludarabine-based chemotherapy, 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses (CR and PR), while the remainder included the same number of patients with stable disease (SD) and progressive disease (PD) (5, 9.6%). Most of the patients (42, 78%) relapsed during the follow-up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overall survival was 76 months. In the group of patients who received FC in the first treatment line (43/54), median expression of SLC28A3 mRNA in patients who experienced CR, PR, SD, and PD was 0.036±0.030, 0.062±0.063, 0.033±0.025 and 0.157±0.257, respectively. The level of SLC28A3 expression was not associated with the IGHV mutational status. Patients who experienced PD to FC treatment overexpressed gene for hCNT3 compared to patients who achieved CR (p=0.013) and PR (p=0.05). We detected a significantly higher level of SLC28A3 expression in patients who experienced PD to FC treatment in comparison to patients who achieved CR (p=0.013) and PR (p=0.05).

Summary/Conclusions: Overexpression of SLC28A3 gene is a predictor of resistance to treatment with FC chemotherapy. Further studies are warranted to confirm these findings.

PB1772
THE SPECTRUM OF TP53, SF3B1, AND NOTCH1 MUTATIONS IN CHRONIC LYMPHOCYTIC 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 IGVH1-69 and IGVH3-21 (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, suffers 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 radionucleotide 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 hotspots regions of these genes were the vast majority of CLL-specific lesions were reported: in c.711 (p.V237I), c.2114 (p.A705T) of NOTCH1 gene, and in exons 14, 15 and 16 of SF3B1 gene, correspondingly.

Results: We found TP53 and SF3B1 mutations with similar incidence in both groups – in 11.3% and 10.0% of IR-exposed patients, and in 12.7% and 11.5% of IR non-exposed CLL patients, respectively. In contrast, NOTCH1 mutations were found in IR-exposed patients with lower frequency in IR-exposed patients in comparison with the control group (6.7% vs 17.7%; p=0.012). Some other features were found among IR-exposed CLL patients also. Specifically, TP53 mutations were seen with equal frequency among mutated (11.1%) and 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 SF3B1 and TP53 lesions (p=0.001 in comparison between observed groups). Among IR-exposed CLL patients we found two different cases with identical rare mutation of TP53 gene - c.665C>T substitution leading to change proline for leucine at codon 222 (Pro222Leu). This substitution is very likely to represent inherited TP53 mutation, which may influence CLL development under IR exposure.

Summary/Conclusions: In summary, our data suggest that TP53 abnormalities are involved in CLL development in sufferers of the Chornobyl NPP accident and also a possible interaction between inherited IR sensitivity caused by mutation in TP53, radiation and CLL development.

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 clinical event evolving into tumor heterogeneity. We focus on Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma and Follicular lymphoma (FL) that are currently considered incurable. Though current treatment regimen is based on CHOP treatments in CLL and MM cancer eventually relapse. Current challenges in using therapeutics against CLL and MM includes design of optimal treatment for individual patients based on characterization the tumor and its intratumor heterogeneity as observed by whole genome sequencing. Efficient therapies require a personalized approach that combines targeting lymphoma cells and the tumor microenvironment by restoring the patient’s own anti-tumor immunity. One solution to this challenge is the so-called “n-of-one” studies where protocols are organized with diagnostically based patient stratification to individualized treatment (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 APRIL and BAFF for 24 hours stimulation. We perform drug sensitivity screening with Prestimulated CLL cells in 384 well formats without feeder cells. We culture MM cells in 384 well format for drug screening in response to Thelper Cells prestimulation in the presence of IL2. To support high-throughput drug sensitivity screening. We use cell-based assays such as CellTiter-Glo® for 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).

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 xenografting and “n-of-one” clinical trial studies.
MGEDONIAN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS PATTERN AND STEREOTYPED RECEPTORS REPERTOIRE OF MUTATIONAL STATUS, IMMUNOGLOBULIN HEAVY VARIABLE GENES

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Background: Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in Western world with highly variable clinical outcome. Rituximab is a monoclonal chimeric anti-CD20 agent, that has demonstrated significant benefit for patients with different form of B cell lymphoproliferative disorders. Chemoinmunotherapy with rituximab, fludarabine and cyclophosphamide (R-FC) has shown to prolong progression free survival (PFS) and overall survival in CLL patients compared with chemotherapy alone. FCGR2A is polymorphic and has two alleles, FCGR2A-131H and FCGR2A-131R. This polymorphic variation is due to a single base substitution of nucleotide adenine for guanine in position 494. FCGR2A-H131 allele has a higher affinity for human IgG2, comparing to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: 158 valine (V158) and phenylalanine (F158) due to single base substitution of tautomine to guanine at position 559. FCGR3A-158V variant has higher affinity for Fc gamma receptor than 158F variant. These Fc gamma receptor polymorphisms may influence antibody-dependent cytotoxicity (ADCC), complement-dependent 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. Mean age of our patients was 62.7 (36.7-88) and 63% were female. 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)x10^9/L. Percentage of previously treated patients was 51/90 (56.6%). Average numbers of R-FC cycles were 4.3 and median PFS was 35.1 months. Median time of observation after treatment was 3.6 years (range: 6 months-8 years). Response was evaluated 2 months after therapy according to National Cancer Institute (NCI) criteria. Complete response (CR) was achieved in 24/90 (26.7%), partial response (PR) in 56/90 (62.2%) and no response in 10/90 (11.1%). DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods. The distribution of genotypes was compared by using a chi-squared test or Fisher’s exact test.

Results: The distribution of genotypes in our patients was: 33% H/H, 49% H/R and 18% R/R for FCGR2A and 43% V/V, 40% V/F and 17% F/F for FCGR3A. Rate of CR and PR were similar irrespective of the FCGR variants and our results did not demonstrate significantly different genotype distribution for FCGR2A (p>0.001) or FCGR3A (p=0.1019) in CLL patients with complete, partial or no response to R-FC therapy (Table 1).

Table 1. Genotype distributions for FCGR2A and FCGR3A in patients with CLL.

<table>
<thead>
<tr>
<th>FCGR2A/FCGR3A</th>
<th>Complete Response n=24 (26.7%)</th>
<th>Partial Response n=56 (62.2%)</th>
<th>No Response n=9 (11.1%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCGR2A 131H/131R (131H/H)</td>
<td>8 (20.8%)</td>
<td>26 (46.4%)</td>
<td>0 (0%)</td>
<td>0.0580</td>
</tr>
<tr>
<td>FCGR2A 131H/131R (131H/R)</td>
<td>6 (15.6%)</td>
<td>20 (35.7%)</td>
<td>1 (1.1%)</td>
<td>0.0580</td>
</tr>
<tr>
<td>FCGR2A 131H/131R (131R/R)</td>
<td>0 (0%)</td>
<td>10 (17.2%)</td>
<td>2 (2.2%)</td>
<td>0.0580</td>
</tr>
<tr>
<td>FCGR3A 158V/158V (158V/V)</td>
<td>8 (20.5%)</td>
<td>27 (45.0%)</td>
<td>5 (8.3%)</td>
<td>0.0109</td>
</tr>
<tr>
<td>FCGR3A 158V/158V (158F/V)</td>
<td>10 (26.3%)</td>
<td>33 (55.0%)</td>
<td>7 (11.7%)</td>
<td>0.0109</td>
</tr>
<tr>
<td>FCGR3A 158V/158V (158F/F)</td>
<td>10 (26.3%)</td>
<td>33 (55.0%)</td>
<td>7 (11.7%)</td>
<td>0.0109</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our results are similar with previously published reported results in other studies in CLL patients, but in contrast with the results for follicular lymphoma (FL), which showed that high-affinity FCGR2A-131R/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.

PB1777

MUTATIONAL STATUS, IMMUNOGLOBULIN HEAVY VARIABLE GENES PATTERN AND STEREOTYPED RECEPTORS REPERTOIRE OF MACEDONIAN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: The mutational status of the immunoglobulin heavy variable (IGHV) genes is established as one of the most important prognostic molecular genetic markers in chronic lymphocytic leukemia (CLL). It divides the CLL patients into two subsets with a different clinical course, mutated (M-CLL) and unmutated (U-CLL). U-CLL is delineated with a cutoff value of 98% identity with the closest germ line of IGHV genes. The shuffling of the CLL IGHV gene followed by rearrangement on a different genetic background is shown to play an important role in antigen stimulation in the pathogenesis of the CLL disease. In addition, a strong bias in the use of individual genes and subgroups between normal and malignant B-cells and presence of highly homologous “sterotyped” heavy complementary-determining region 3 (VDJCD3) is shown, which suggests the role of a specific antigen in the pathogenesis of the disease.

Aims: In this study, we analyzed the mutuation 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 IGHV genes was determined using two databases: IMGT/TV-QUEST tool and igBLAST software. The stereotyped subset assignment was performed using ARRest/AssignSubset 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 subgroup (44.3%), IGHV4 (23.7%), IGHV5 (2.0%), and IGHV2 (2.0%). 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 IGHV 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 IGHD4 (46.9%) and IGHD3 was not expressed. IGHD4 (46.9%) and IGHD3 (27.3%) showed a statistically significant difference between the groups (p<0.01). The distribution of IGHD subgroups was as follows: IGHD1, 42.5%; IGHD2, 20.4%, IGHD3, 15.8% and IGHD4, 15.8%.

Summary/Conclusions: Our study showed a strong correlation between IGHV gene mutational status and clinical course of CLL. Results on IGHV-IGHD-IGHJ gene 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 IGHV 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.
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: Prognostication is not standardized in HCL, emphasizing the relevance of the characterization of HCL populations.

Methods: We retrospectively analysed 40 patients (90% men), diagnosed between 1997 and 2016, with a median follow-up of 6 years.

Results: At presentation, the median age was 58 years and 69% of patients were symptomatic - fatigue (53%), B symptoms (50%), bleeding (14%), abdominal discomfort (6%) and severe infection (22%). The commonest cytopenia was thrombocytopenia (70%), with median platelet count being 66x10^9/L. Monocyte counts below 0.1x10^9/L were observed in 61% of patients. Splenomegaly was observed in 83% of the patients and 21% had abdominal lymphadenopathies. The majority of the (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. Treatment was required in 33% of the patients, of which the majority received cladribine. The median time-to-next-treatment (TN7) from first to second line treatment was 91%, 50% achieving CR, of which 33% were classified as MRD-negative CR. Only 5% of the patients required further treatment lines. Even the presence of scarce hairy cells in the bone marrow precluded classification of response as CR. This might have contributed to the low CR levels observed in our patients. As post-treatment bone marrow biopsies were available in only 24 patients, response analysis was restricted to these patients. All of these 24 patients had bone marrow fibrosis at diagnosis, which reverted when and in whom first CR was obtained. Median overall survival (OS) was not reached and, at 10 years, the OS was 90%. Four deaths occurred, all unrelated to HCL. Regarding prognosis, 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 kappa (k) light-chain restriction (LCR) displayed a significantly higher TFS than the 39% without lambda (λ) LCR (p=0.03, Wilcoxon-Gehan test). To the best of our knowledge, there are no published reports on prognostic value of LCR in HCL (Figure 1).

Summary/Conclusions: Monoclonal B-cell lymphocytosis (MBL) is characterized by the presence of ≤5X10⁹ 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 staining and the screening for >5x10⁹ total PB leukocytes. 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 imbalance of sigk: sigl ratio of >3:1 or >1:3, were further tested for CDS, CD23, CD20 and CD79a expression.

Results: Of total 616 subjects, MBL was found in 8 cases (1.2%) including 3 and 5 female and male cases respectively. Among 40 years or older, MBL was found in 5 out of 448 cases (1.1%). Compared with non-MBL group, subjects were significantly younger (median age 49 years versus 55 years; p=0.01) and had a significant higher number of absolute and B lymphocyte count (median 3.1 versus 1.6 X10⁹/L; p=0.03 and 0.35 versus 0.16 X10⁹/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 family 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 (<0.5X10⁹ clonal B-cells/L) while only 2 cases had high-count MBL (>0.5X10⁹ clonal B-cells/L). All 8 cases had persistent positivity of MBL clone after tested was repeated within 3 months after the initial test. In the 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+ and dim and light chain restriction). Whereas 1 case had atypical CLL phenotype MBL (CD5+, CD23-, CD20+ and dim light chain restriction) but CD23+ and 1 case had non-CLL phenotype MBL (CD20+ but CD5-). In univariate analysis, age (RR 4.19; 95%CI 1.0-17.7; p=0.049), absolute lymphocyte count (RR 2.76; 95%CI 1.04-8.87; p=0.047), family history of LPD (RR 122; 95%CI 51.1-293.4; p<0.001) and

Figure 1.

Summary/Conclusions: If multicentre studies corroborate our findings, LCR may be of use in the prognostication/risk stratification of HCL. Similarly with multiple myeloma and other hematological malignancies, lambda (λ) LCR appears to correlate with worse prognosis, leading to a shorter TFS.

PB1777

CLINICAL EFFICACY AND LONG-TERM OUTCOMES OF SPLENECTOMY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is often accompanied by splenomegaly, which can enlarge to a giant size, causing abdominal discomfort, regional portal hypertension, and becomes a place of malignant cells concentration. In 2.3-4.3% of cases CLL may be complicated by autoimmune cytopenias (autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), Evans-Fisher syndrome). Accordingly, the effectiveness of steroid and chemotherapy in such cases may be impaired, raising the question of splenectomy advisability.

Aims: To analyze splenectomy effectiveness in patients with CLL.

Methods: Splenectomy was performed in 41 patients with CLL, 12 of which were patients with CLL and ITP, 9 with CLL and warm type AIHA, 5 patients with CLL and Evans-Fisher syndrome, along with 18 CLL patients without immune disorders. Among the patients there were 26 males and 15 females. Indications to splenectomy were following: massive splenomegaly with abdominal discomfort, immune cytopenia and regional portal hypertension. In one female patient the surgical intervention was performed urgently due to spontaneous splenic rupture and acute intra-abdominal bleeding.

Results: Splenectomy was effective in 37 patients (90.2%): abdominal discomfort disappeared, hemolysis stopped and hemoglobin levels normalized or increased, platelets numbers normalized or increased. Splenectomy was ineffective in 3 patients with CLL associated with ITP: amid elimination of abdominal discomfort the platelets number did not increase significantly (2 patients), while in 1 patient despite increase in platelets number leukemia progression was observed. One (2.4%) patient with CLL and AIHA died on 3rd day after surgery because of acute adrenal insufficiency. The analysis of late effects of splenectomy in patients with CLL showed that average life expectancy after the surgery comprised 111.6 months within observation period between 11 and 277 months. In patients with CLL with immune cytopenias the average life expectancy after surgery was shorter and equal to 60.7 months within the observation period between 2 and 361 months.

Summary/Conclusions: Splenectomy remains an effective method of treatment of patients with CLL. Companied by severe splenomegaly and immune cytopenia. Long-term results of splenectomy in patients with CLL without cytopenia are better than in patients with CLL and cytopenias. Aggressive hemolysis, large spleen covered in perisplenic adhesions, amid portal hypertension and thrombocytopenia are considered to be special surgical risk factors in this patients.

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influenza vaccination (RR 10.47; 95% CI 2.54-43.07; p = 0.003) were associated with increase risk of developing MBL. After adjusted for age, only history of influenza vaccination and family history of LPD were an independent risk factor for developing MBL with age adjusted RR of 9.75 (95% CI 2.3-40.5; p = 0.002) and 92 (95% CI 56.3-149.5; p<0.001), respectively.

Summary/Conclusions: MBL prevalence in Thai population is much lower than previously reported. It more frequent in elders and associated with family history of LPD and influenza vaccination. Although uncommon, the presence of high-count MBL warrants further investigations to define the biological and clinical significance in term of LPD transformation and long-term survival.

PB1779 SPONTANEOUS CLINICAL REGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 9 CASES FROM THE ERIC REGISTRY
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Background: Spontaneous clinical regression in chronic lymphocytic leukemia (CLL) is rare (1% per year). We previously reported on the clinico-biological features of 9 Binet stage A CLL patients from our Center in Rome who experienced a persistent spontaneous clinical regression of the disease at a median time of 11 years to the last follow-up. In 5/9 cases, it has been possible to follow-up. The lymphocyte count at CLL regression was 3.16 x 10^9/L (1.3-4.9, with a persistent small CLL clone (CD19+CD5+CD23+light chain restricted: 44%, range 5-60%). Biologic features included negative CD38, mutated IGHV in 8/8 with VH3-30 (2), VH3-21, VH3-15, VH3-23, VH4-31, Vk4-1 usage, and a distinctive gene expression profile.

Aims: To conduct a retrospective collection of clinical data and basic biologic information on CLL spontaneous regressions and to make them accessible for future research.

Methods: A registry of spontaneous CLL regressions (absence of lymphadenopathy, splenomegaly as constitutional symptoms, peripheral blood (PB) lymphocytes <4 x 10^9/L in the absence of any previous treatment) was launched within the ERIC consortium.

Results: So far, 9 CLL patients showing a spontaneous regression have been reported and 8 have been formally registered, 7 from Italy and 2 from Sweden. Six cases were males and 3 females, with a median age of 57 years at diagnosis (range 51-82), stage Binet/Rai A/I in 6, A/I in 2 and B1 in 1. The median lymphocyte count at diagnosis was 14.1 x 10^9/L (5.3-51.9). Biologic features included: mutated IGHV in 8/8 with VH3-30 (2), VH23-1, VH13, 15, VH3-24, VH-31, VH-43, VH-44, VH-59; CD38- <30% in 6/8; ZAP70 <20% in 4/6; FISH (7 cases): mutated IGHV in 8/8 with VH3-30 (2), VH3-21, VH3-15, VH3-23, VH4-31, mutated TP53.

Summary/Conclusions: We studied 9 cases presenting spontaneous clinical regression and characterized the distinctive gene expression profile.

PB1780 CLINICAL AND LABORATORY CHARACTERIZATION OF PLATELET DYSFUNCTION DURING IBRUTINIB TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. MONOCENTRIC EXPERIENCE
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Background: Ibrutinib (IB) is a potent and irreversible inhibitor of Bruton’s tyrosine kinase (Btk) approved by FDA for the treatment of patients (pts) affected by chronic lymphocytic leukemia (CLL) with del 17p or TP53 mutation or for pts with relapsed/refractory (R/R) CLL. IB is associated with bleeding events usually mild (Common Toxicity Criteria (CTC) grades 1-2), rarely severe (grade 3-4), A defect of platelet function, namely an inhibition of Btk-mediated signaling by platelet glycoproteins (GP) GPVI and GPIb, has been hypothesized to cause these bleedings. IBR associated bleedings and platelet dysfunction may be relevant in CLL pts who are usually elderly and with comorbidities requiring antithrombotic therapies.

Aims: To investigate and characterize the effect of IBR on platelet function in pts with CLL.

Methods: We enrolled from May 2014 to December 2016 twenty pts with CLL treated with orally administered 420 mg daily of IBR; 18 R/R CLL pts received IBR in monotherapy and 2 pts with previously untreated CLL received IBR as induction with anti-G-CSF MoAb. Median age was 68 years (57-84); 13 pts had unmutated IgVH and 2 had 17p deletion. The median number of prior therapies in R/R CLL pts was 3 (2-7). Five pts discontinued IBR therapy: 2 for Richter’s transformation, 1 for progressive CLL, 1 underwent alloengenic HSCT, 1 for heart disease. The platelet function was studied before starting IBR and during IBR treatment with using platelet-rich plasma and the following agonists: ADP 2-4 uM, PAR1-AP 25 uM, Collagen 10-3.3-2 ug/mL, arachidonic acid 1 mM, ristocetin 0.6-1.2 mg/mL. Also measurements of von Willebrand factor antigen (vWF:Ag) and ristocetin cofactor activities (RiCo) by chemiluminescent immunoassay were performed. All pts had measurements of the platelet function at the baseline and after 1, 3, 6 months initiation of IBR and then every 3 months up to 24 months. Median observation period was 9 months. No patient received concomitant antiplatelet or anticoagulation therapy.

Results: Nineteen pts achieved a partial response and an increase of hemoglobin and platelet count, 2 pts with previously untreated CLL and 12 in 1 case. No patient needed IBR interruption or dose reduction. All pts displayed severe impairment of collagen induced aggregation upon IBR. Reduction of maximal aggregation (35.6% vs 70.6% baseline) and prolongation of the lag phase (261 +/- 34 sec vs 72 +/- 26.8 sec baseline) by 2 uM collagen was measured in all pts during IBR. In 10 pts a significant improvement of the aggregation by 2 uM ADP (71 +/- 31.8% vs basal 48.6%+/-31%) and 4 uM ADP (84 +/- 11% vs basal 64% +/-25%) was found during IBR. The aggregation by 25 uM PAR1-AP, 1.2 mg/ml ristocetin and 1 mM arachidonic acid was unchanged during IBR. All pts had measurements of the platelet function at the baseline and after 1, 3, 6 months initiation of IBR and then every 3 months up to 24 months. Median observation period was 9 months. No patient received concomitant antiplatelet or anticoagulation therapy.

Summary/Conclusions: Our study showed minor bleedings in pts treated with IBR. A severe impairment of collagen-induced aggregation was caused by IBR treatment and was countiracted by leukocyte lysis. The baseline platelet function profile, that could explain, at least partially, the mild clinical phenotype in treated pts. The assessment of platelet function in IBR treated CLL pts could help to predict and monitor the bleeding risk, and to guide pts through invasive procedures. In addition, pts under anticoagulant or antiplatelet treatment might need be carefully monitored by clinical and laboratory evaluation.

PB1781 HAIRY CELL LEUKEMIA: A SUMMARY OF CLINICAL DATA ON 202 PATIENTS AND THE RESULTS OF THERAPY WITH CLADIBRINE IN ISRAEL
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Background: Hairy cell leukemia (HCL) accounts for approximately 2% of all leukemias and is associated with pancytopenia, splenomegaly, and recurrent infections. Therapy with the purine analogues cladribine (2CdA) or pentostatin (2’deoxycoformycin), has been most effective and both agents have achieved equivalent results in HCL. In this regard cladribine given as a single course, achieves a high response rate. Several alternative dosing schedules have been reported, mostly in subcutaneous (SC) or oral therapy. Response may 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 have summarized the Israeli experience with HCL over the past 30 years, and analyzed demographic data, relevant laboratory and clinical parameters with special emphasis on outcome after first line treatment with cladribine.

Methods: We collected retrospective data on patients with HCL from 12 medical centers in Israel, followed and treated during 1985-2015. The study was approved by local institutional IRBs of each medical center.

Results: Data from the medical records of 202 patients with HCL was summarized. Mean follow up was 7.5 years (0.1-40), with a 5 and 10 years’ overall survival of 96% and 80.62%, 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 (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 11% 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 than 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 (0-45) (p=0.05), and the length of NADIR was 18 and 20 days for IV and SC delivery respectively (p=0.33).

Summary/Conclusions: This study is the first comprehensive summary of the natural history of 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 group (PR Binet A) who achieved at any time during the course of the disease, using the time when a remission was achieved according to IWCLL recommendations and by changes in clinical stage.

Changes in clinical stage provide reliable information on the degree of response to therapy in patients with CLL, particularly those in the IWCLL PR category. This study supports the use of clinical stages as a complementary and simple tool to assess response in patients with CLL, both at the end and over the course of treatment.

Summary/Conclusions: Changes in clinical stage provide reliable information on the degree of response to therapy in patients with CLL, particularly those in the IWCLL PR category. This study supports the use of clinical stages as a complementary and simple tool to assess response in patients with CLL, both at the end and over the course of treatment.

PB1783

INCIDENCE OF THYROID GLAND DISORDERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Frequency of autoimmune complications like immune anaemia 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 scintographies of CLL patients were performed. Demographic data, Rai-stages, and established thyroid disorders were recorded.

Results: One hundred (110) CLL patients were included into the study (65 male, mean age was 62±10.4 years). 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 sickness 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=0.999, p=0.167, respectively). Anti-Tg positivity was also 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 11 patients in ≥65 years old age group (p=0.053). There was no statistically significant difference in thyroid function tests according to the Rai-stages, ages and sexes.

**Summary/Conclusions:** We determined that incidence of hypothyroidism 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—in 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 leukemia. 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 is 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 selected group of SLL pts diagnosed in our centers b)correlate clinicopathological characteristics and treatment with response and survival c)detect possible differences in terms of response and survival between SLL pts according to LN characteristics (size of LN and presence of PCs)

**Methods:** Pts diagnosed with SLL from 2007 up to now fulfilling the diagnostic criteria of SLL(LS) were included. Clinical and biological data were recorded at diagnosis as well as treatment related variables, such as type of treatment, response and patient survival. Moreover, LN features such as the size, and the presence of PCs were also studied. PCs were identified in hematoxylin and eosin sections and defined as pale areas containing polyclonality and paraimmunoblasts, surrounded by a dark background of small lymphocytes.

**Results:** 47 pts were analysed. Pts’ median age was 69y (range, 40–87) with no gender predominance (24male/23female). According to Binet staging system 12,47 pts were E, 31 pts were F. According to Rai staging system, 12 pts were Stage I,9 pts were Stage II, and 26 pts were Stage III. According to Binet staging system 12,47 pts were E, 31 pts were F. According to Rai staging system, 12 pts were Stage I,9 pts were Stage II, and 26 pts were Stage III. Regarding type of treatment, 47 pts were under treatment during the questionnaires filling period, showed a higher adherence compared to those who interrupted questionnaires. At this timepoint we cannot yet evaluate system effectiveness as the study is still ongoing. However, we determined that pts under treatment during the questionnaires administration period, showed a higher adherence compared to those who interrupted questionnaires.

**Conclusion:** We determined incidence of hypothyroidism 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—in both sexes and all Rai-stages should be examined for thyroid gland disorders, mainly for Hashimoto thyroiditis.
Background: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in adults in western 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 patients were 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 3.60 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, et al., 2018) for treatment initiation, assessment of response 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 cells. The maintenance therapy was conducted for 2 years; Ibrutinib 420 mg, orally, daily (n=28) continuously. The remaining patients with MT of Rituximab, compared with ibritinib: 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 MRD-positive CR – 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 of MT 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 ONEFLOW LYMPHOCYTES SCREENING TUBE
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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, setup reagents, and protocols. The BD OneFlow LST (Lymphoid Screening Tube) is intended for flow-cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T, and NK lineages in specimens (peripheral blood, bone marrow, and lymph node) from patients with hematological disorders. BD OneFlow LST acquisition and analysis template version 1.0 was revised to version 2.0 to include determination of lymphocytes as a percentage of total leukocytes. The FCS files from evaluable specimens of the original LST clinical trial were reprocessed using BD OneFlow LST template v2.0.

Aims: The objective of this study was to assess the FCS files from all the evaluable specimens previously collected using LST template v1.0 in the original clinical study to demonstrate equivalency between the investigational BD OneFlow LST system and the comparator EF liquid reagent system on a BD FACSCanto II flow cytometer with the 4-2H-2V CE-IVD configuration and LST template 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 specimens in the original study were simultaneously stained with investigational BD OneFlow LST and comparator EF liquid reagents within 24 hours of collection and were acquired within 60 minutes of staining. In the current study, analyses were performed on a BD FACSCTojo II instrument using LST v2.0 templates and BD FACSDIVA software v6.0.1. Forward and side scatter parameters were categorized as normal or follow-up needed. If follow-up was needed, specimens were categorized as B-, T-, NK-, or other-cell lineage. Overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For quantitative (percent) comparison of defined cell populations, Deming regression (slope and intercept analysis) was performed between the BD OneFlow method and the EF method.

Results: The BD OneFlow LST system compared to the EF system gave 100% (207 of 207) overall agreement (lower 95% CI: 98.6%) in delineating patients into normal (no follow-up) or follow-up, and 100% overall agreement in identifying B-, T-, NK-, and other-cell lineages with 96% CI: 98.8%. There was 100% positive agreement and 100% negative agreement between BD OneFlow and EF for follow-up vs no-follow-up (normal) and for all cell lineages from specimens that required follow-up. Furthermore, compared to the ultimate BD system, the BD OneFlow LST system met the acceptance criteria for the quantitation of cell samples (excluding regression) for the defined cell populations.

Summary/Conclusions: The multisite performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was concordant in identifying abnormal from normal mature populations in patients with CLPDs. BD OneFlow LST is fit for in Vitro Diagnostic Use. CE Marked to the European in Vitro Diagnostic Medical Device Directive 98/79/EC: 23-19566-06.

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. Hypogammaglobulinaemia 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 (hevylight) 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. Sample analyses were performed for multiple analytes: IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgA lambda, IgM lambda and Free light chain: kappa (K) and lambda (L) ratio of K/L and calculation of ratios of monoclonal/polyclonal immunoglobulin (HLC ratio).

Results: The total cohort consisted of 126 “treatment - naïve”, patients with CLL and 26 healthy volunteers. Median age was 64 years, 64% were males and 78% had Binet stage A, while 19% and 3% were stages B or C respectively. Significantly higher HLC ratio was found in patients with Binet stage A (median age - 47 years) compared to healthy volunteers (median age - 50 years; p=0.002). There was a trend towards higher HLC ratio in patients with Binet stage B (p=0.028). There was 100% positive agreement and 100% negative agreement between BD OneFlow and EF for follow-up vs no-follow-up (normal) and for all cell lineages from specimens that required follow-up. Furthermore, compared to the ultimate BD system, the BD OneFlow LST system met the acceptance criteria for the quantitation of cell samples (excluding regression) for the defined cell populations.

Summary/Conclusions: The multisite performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was concordant in identifying abnormal from normal mature populations in patients with CLPDs. BD OneFlow LST is fit for in Vitro Diagnostic Use. CE Marked to the European in Vitro Diagnostic Medical Device Directive 98/79/EC: 23-19566-06.

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 prognostic value of cytokines in treatment of patients (pts) with hairy cell leukemia (HCL) are not finally 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 IFNo 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 estimated 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 (8.56 pg/ml; p=0.275), however levels of IFNo 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 and 1568.22 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. Therap- y with IFNo 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=0.15; p=0.01). There was no correlation of amount of lympho- cytes in peripheral blood of HCL pts (r=0.24; p=0.09). Median serum concentra- tion of sIL-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 sIL-2R level in serum (r=0.27; p=0.08). Obtained results may be an evidence of pre- dominant secretion of sIL-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 IFNo or 2-CdA treatment a reliable relationship was revealed, which is possible to use for prediction of clinical course of this disease. Moreover sIL-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 is 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 24 oncologists/hematologists in the United States. Oncologists/hematologists provided patient level clinical data obtained from patient charts among CLL patients who initiated first-line therapy for CLL between January 2010 and December 2014. Selected patients were categorized into 2 cohorts based on their best response: patients who achieved CR and patients who did not achieve CR (non-CR). The non-CR cohort included patients with partial remis- sion (PR), stable disease (SD) and progressive disease (PD). iwCLL 2008 cri- teria 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 with SD, and 6 with PD). Most patients were male, in their early sixties, and died (unadjusted HR=2.61, p<0.05) compared to patients in the CR cohort. Furthermore, significantly better outcomes were observed in 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 developed 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 immobilized on plastic coverslips in spots 2 mm in diameter. In order to study the peripheral blood mononuclear cells (PBMC) for 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. The microarray was compared to 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 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-progression/died. Patients who did not achieve MRD- had an over three-fold higher hazard of progression/death compared to patients who achieved MRD- (adjusted HR=3.75, p<0.05). No death events were observed among patients who achieved MRD- while 4 (6%) events were observed among those who did not achieve MRD-.

Table 1.

PB1793

COMPARATIVE ANALYSIS OF INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA, PROGRESSION-RISK SCORE AND MD ANDERSON CANCER CENTER 2011 SCORE: REAL WORLD DATA FROM A SINGLE INSTITUTION

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Background: In recent times, several powerful prognostic scores have been developed in order to predict to first treatment (TTFT) and overall survival (OS) of patients with chronic lymphocytic leukemia (CLL). The international prognostic index for chronic lymphocytic leukemia (CLL-IPI) developed by The International CLL-IPI working group was found to predict OS and TTFT, while the rest of two scores- progression-risk score (PRS) and MD Anderson Cancer Center Score 2011 (MDACC 2011) have been developed for prediction of TTFT in early stage CLL patients.

Aims: The aim of this study was to compare the CLL-IPI, PRS and MDACC 2011 prognostic scores based on their impact on TTFT, treatment response (TR), progression-free survival (PFS) and OS of 54 treated CLL patients.

Methods: We retrospectively analyzed data from 54 consecutive CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia from 2003 to 2013. Blood samples were prospectively collected and analyzed for biological and molecular features (IGHV, FISH and TP53), as well as standard laboratory parameters. The three scores were retrospectively calculated using formulas from the original articles (International CLL-IPI working group, Lancet Oncol 2008, for CLL-IPI; OncoL 2015, for PRS; and Wierda et al, J Clin Oncol 2011, for MDACC 2011 score), and, then, correlated with TTFT, TR, PFS and OS of patients from the studied cohort.

Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with 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. Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overall survival was 76 months. Lower score values for all the three scoring system (CLL-IPI, PRS, and MDACC 2011) were found to be significant (p=0.037, RR=1.5, 95%CI 1.02-1.81) for OS. Cox regression analysis revealed that CLL-IPI and PRS are significant predictors of TTFT (p=0.003, RR=1.4, 95%CI 1.1-1.7 and p=0.019, RR=1.4, 95%CI 1.1-1.9, respectively), while MDACC 2011 was found to be borderline significance (p=0.052). In the multivariable analysis PRS emerged as the most significant predictors of TTFT among the three examined scores (p=0.041, RR=1.35, 95%CI 1.01-1.81). Regarding TR, only PNS appeared to have borderline statistical significance (p=0.052), showing that patients with lower score value may achieve better TR. Lower CLL-IPI can predict longer PFS after the first line treatment (p=0.007, RR=1.7, 95%CI 1.2-2.57), as well as PRS (p=0.039, RR=1.5, 95%CI 1.03-3.1). MDACC 2011 has not shown to have influence on PFS. Multivariable analysis confirmed PRS to have the strongest predictive value of all the three scores regarding duration of PFS (p=0.039, RR=1.8, 95%CI 1.02-3.1). Furthermore, CLL-IPI and PRS were found to be significant predictors of OS (p=0.005, RR=1.4, 95%CI 1.1-1.8 and p=0.037, RR=1.5, 95%CI 1.1-2.0). Only CLL-IPI was significant predictors of TTFT, as well as of duration of TR and OS. Further studies are warranted to confirm these findings.
MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS T1 (B-CLPD T1) PANEL
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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 B-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 the BD OneFlow LST for the immunophenotyping of B cells and distinguishing chronic lymphocytic leukemia (CLL) from other B-CLPDs such as atypical CLL, follicular cell lymphoma, mantle cell lymphoma, etc.

Aims: The objective of this study was to demonstrate equivalency (accuracy) between the investigational BD OneFlow LST and BD OneFlow B-CLPD T1 system and the corresponding comparator EF liquid reagent system on the BD FACScantor II flow cytometer using BD FACsDiva software.

Methods: De-identified remnant peripheral blood (PB) (n=70) and bone marrow (BM) (n=31) patient specimens were collected in EDTA or heparin anticoagulants at four external study sites and tested within 26 hours of draw. Informed consent was not required in this clinical study. Specimens were stained with BD OneFlow LST in combination with BD OneFlow B-CLPD T1 tubes and comparator EF liquid reagents. Acquisition and analysis were performed on a BD FACScantor II instrument using BD OneFlow LST and BD-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 of CD45+CD19+ aberrant cell populations, overall agreement with one-sided lower 95% confidence limits was calculated.

Results: All evaluable specimens were identified by the OneFlow LST as having B-cell populations requiring follow-up by both methods. Compared to the EF system, the BD OneFlow LST in combination with the BD OneFlow B-CLPD T1 system had 100% (101 of 101) overall agreement in classifying patients as having CLL (54 of 54 concordant) and in identifying patients with other B-CLPD diseases (47 out of 47 concordant) with a lower 95% CI of the overall agreement of 97.4%. The BD OneFlow B-CLPD T1 system, compared to the EF system, gave 100% (101 of 101) concordant agreement for the qualitative assessment of the relative fluorescence intensity of CD45+CD19+ aberrant cell populations for CD20+, CD200+, and CD23+ subsets and 99.1% agreement for the CD79b+ subset.

Conclusion: The multisite performance evaluation between the BD OneFlow system (LST and B-CLPD T1) and the comparator EF liquid reagent system in determining in distinct B-cell populations of normal and abnormal B-cell populations 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.

PB1795
COMBINED PATTERNS OF IGHV REPERTOIRE AND MOLECULAR ALTERATIONS IN CHRONIC LYMPHOCYTIC LEUKAEMIA- SINGLE CENTER EXPERIENCE
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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.

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 IGL-associated IGHV gene repertoire (IGHV61-69 associated with HD3 gene and HJ6 gene). Another group, frequently associated with complex karyotypes and poor-prognosis cytogenetic alterations, a second group enhanced in clones expressing specific IGHV subgroups (IGHV3-23 associated with HD2 genes and HJ6 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 different geographic and environmental circumstances are required to validate our findings and discard or confirm the potential role of some antigen-binding BCR specificities contributing to clonal evolution.
Background: Constitutive activation of B-cell receptor signalling appears to be essential for the proliferation of malignant B cells. Bruton’s tyrosine kinase (BTK) has been identified as an essential component of the B-cell receptor signaling 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. During the period 2015 were analyzed for this study and were categorized into two groups (1)

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 relapse was 10 months for patients with B-CLL and 6.5 months for patients with MCL. The median number of comorbidities 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 treated by R-CTX was more than 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 had and without >7 p53 deletion. 11/28 patients experienced side effects: 8 had grade 1 and 2 effects (diaphoresis, 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 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 a lower risk of bacterial and viral overall survival. Moreover, patients who received fewer lines of prior treatment had a better survival. Patients with >1 p53p deletions in MCL responded as well as 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
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Background: The addition of the monoclonal antibody rituximab to chemotherapy (R-CHOP) 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, willingness to participate and various in- and exclusion criteria. To date, the efficacy of rituximab added to standard chemotherapy in first line and relapsed CLL patients has been poorly evaluated in observational studies in unselcted real-world CLL patients.

Aims: To evaluate the efficacy of rituximab-chemotherapy (R-CTX) compared to chemotherapy (CTX) in a real-world CLL population.

Methods: All patients from a large teaching hospital diagnosed with immunophenotypically confirmed CLL in the period from 1-1-2000 up to 1-9-2015 were analyzed for this study and were categorized into two groups (1) those treated with CTX and (2) those who received R-CTX. The clinical treatment of patients was evaluated based on the “treatment-free interval” (TFI), defined as the time from stop of chemo/immuno/therapy to start of next treatment. Patients who did not need next treatment were censored at time of last follow-up or death. In addition, overall survival (OS) for patients treated in the period which chemotherapy was the only available therapeutic modality was compared to patients treated before the rituximab era (before and after 1-1-2006, respectively).

Results: A cohort of 375 CLL patients was studied, of whom 124 CLL patients (33%) required treatment in the observation period. The median age at first-line therapy was 67 years; 55% and 45% of these patients received first line CTX or R-CTX, respectively, and 47% of these patients required a second or later line of (R-)CTX. In total 221 treatment periods of (R-)CTX were studied with respect to treatment-free interval, 124 first-line, and 97 courses of retreatment. In the first-line treatment group 12 (10%) and 24 patients (19%) were treated with purine-analogue-based schedules without or with R respectively, i.e. (R-)fludarabine or (R-)fludarabine plus cyclophosphamide, 52 (45%) and 31 patients (25%) were treated with chlorambucil/CVP-based regimens without or with R respectively, and two patients (2%) were treated with CHOP and R-bendamustin. The median TFI for patients treated with CTX was 31 months (95% CI: 20 – 42 months) and was significantly better in the R-CTX group where the median TFI was not reached during the observation time (hazard ratio 0.40, 95% CI 0.22 – 0.73). In second or later lines of treatment 15% and 11 patients (1%) were treated with purine-analogue-based schedules without or with R respectively, i.e. (R-)fludarabine or (R-)fludarabine plus cyclophosphamide, 25 (28%) and 31 patients (32%) were treated with chlorambucil/CVP- based regimens without or with R respectively, and 5 patients (15%) were provided with other treatment modalities, i.e. (R-)CHOP or (R-)bendamustin. The median TFI for CTX was 27 months (95% CI: 18 – 52 months) vs 55 months for R-CTX (95% CI: 41 months – NR), HR 0.47 (95%CI 0.15 – 0.90) for subsequent lines. OS for patients treated in the R era was 48 vs 35 months for patients treated before the introduction of rituximab (p = 0.02).

Summary/Conclusions: Our study shows that the addition of rituximab improved treatment free interval in first- and subsequent lines and prolonged overall survival in a cohort of CLL patients receiving treatment in routine clinical ‘real world’ practice.

Background: Richter syndrome (RS) represents transformation of chronic lymphocytic leukaemia (CLL) or small lymphocytic lymphoma (CLL/SLL) into more aggressive B-cell lymphoproliferative disorder, most commonly, diffuse large B cell lymphoma (DLBCL), vera rarely classical Hodgkin lymphoma (HL). In some point of disease course, 2-10% of all CLL/SLL population develope RS, usually exhibiting chemoresistance and survival within a year after diagnosis.

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 Hematology, Medical Military Academy, Belgrade; Clinic of Hematology, Medical Military Academy, Belgrade, Serbia

Results: In four 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. Pathological 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 3 (8%) of patients were treated with RS-potential treatment as RS. Median time to 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. Eighteen patients received other treatment modalities, such as ESHAP, FC, high dose corticosteroids, COP, RCOP and radiotherapy. After excluding 6 patients with SLL from the group, Cox regression analysis showed that high LDH and low Hb levels at the time of transformation are significant predictors of shorter survival after diagnosis of RS (HR=1.001, 95% CI 1.000- 1.001; p=0.001 and HR=0.978, 95% CI 0.961-0.995; p=0.011, respectively).

Bone marrow as a site of transformation did not reach statistical significance as a predictor of shorter survival after transformation (p=0.087). Median survival after diagnosis of RS was 8 months (range 0-133) (Figure 1).
which are among the main causes of death in this group of patients. The results effects which might occur during therapy. They include infectious complications. The patients (n=3; 6,98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation between the CLL diagnosis and the first appearance of complications during these therapies was considerably lower when compared to people who did not experience any complications. The patients (n=3, 6,98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation between the CLL diagnosis and the first appearance of complications during these therapies was considerably lower when compared to people who did not experience any complications. The patients (n=3, 6,98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation between the CLL diagnosis and the first appearance of complications during these therapies was considerably lower when compared to people who did not experience any complications. The patients (n=3, 6,98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation between the CLL diagnosis and the first appearance of complications during these therapies was considerably lower when compared to people who did not experience any complications. The patients (n=3, 6,98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation between the CLL diagnosis and the first appearance of complications during these therapies was considerably lower when compared to people who did not experience any complications. The patients (n=3, 6,98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group.
Chronic myeloid leukemia - Biology

PB1802
IDENTIFICATION OF NOVEL MUTATIONS IN CANCER-RELATED GENES IN HUMAN ERYTHROLEUKEMIA K562 CELL LINE BY NEXT-GENERATION SEQUENCING
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Background: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by reciprocal chromosomal translocation (t(9;22)(q34;q11)), resulting in the formation of the BCR-ABL fusion oncogene. One of the main modalities of CML in vitro model is the K562 cell line with positive human erythroleukemia cell line derived from a female patient with CML in blastic phase (CML-BP) and representing an important tool for the studies of malignant hematopoiesis in last decades. Although K562 karyotype was described several times, detailed genomic analysis of this cell line is not yet available and to our best knowledge there are no publications yet describing complex genomic landscape of K562 cells.

Aims: The aim of our study was to determine the mutational landscape of K562 cell line using next-generation sequencing (NGS). Additionally classical fluorescence in situ hybridization (FISH) with BCR and ABL1 probes was performed to confirm cytogenetics.

Methods: The K562 cell line was purchased from DSMZ (Braunschweig, Germany). We analyzed almost 1300 genes implicated in human cancer using custom designed capture (SeqCap EZ, NimbleGen, Roche) followed by high-throughput sequencing on Illumina HiSeq 1500. Common variants (>1%) gathered in 1500 and 1000 genes/projects and our internal exome database were filtered out and the subsequent analysis was focused on putative protein damaging variants with the frequency in the database from NHLBI GO exome sequencing project less or equal to 0.01. We used different bioinformatic tools for variant effect prediction (eg. PolyPhen-2, SIFT, IntOGen). Mutations were confirmed with Sanger sequencing. FISH was performed using commercially available probes (Vysis, Abbott, USA), that identifies BCR-ABL1/ABL1-BCR fusion genes.

Results: Sequencing and bioinformatical analysis revealed 88 variants with potential biological significance. We detected Q136fs*13 mutation in TP53, which has already been described in K562 cell line previously by ATCC, but we have also identified several new mutations in genes involved in tumorigenesis and drug resistance (Table 1). Moreover, cytogenetic analysis showed both multiplication of the BCR and ABL1 genes and amplification of the BCR-ABL1 fusion gene (Ph chromosome is present in at least four additional copies).

Table 1. Selected prominent mutations identified in K562 cells.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>NCIH Reference</th>
</tr>
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<tbody>
<tr>
<td>TP53 (q14)</td>
<td>NM_000267.2</td>
</tr>
<tr>
<td>ASXL1 (p.955fs)</td>
<td>NM_03389.5</td>
</tr>
<tr>
<td>KMT2A (p.R3327*)</td>
<td>NM_00247.2</td>
</tr>
<tr>
<td>BRCA2 (p.563fs)</td>
<td>NM_00019.23</td>
</tr>
</tbody>
</table>

Summary/Conclusions: We describe several new mutations in such genes as ASXL1, BRCA1 or MLH1 in one of the most frequently used cell line in leukemia research, K562 erythroleukemia. Our results confirm high level of genomic instability in the blastic phase of CML represented by the K562 cell line and add new, valuable information for researchers who want to employ this cell line. The awareness of the genomic aberrations present in the K562 erythroleukemia cell line is essential for further studies as those aberrations may have a significant impact on the observed results.

PB1803
INVESTIGATION OF POLYMORPHISMS RELATED TO MIR-608 IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expression of the BCR-ABL oncoprotein, which is essential for the pathogenesis of the disease. Imatinib, an ATP-competitive selective inhibitor of BCR-ABL, exhibits unprecedented efficacy for the treatment of CML. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including overexpression of the BCR-ABL gene, the tyrosine kinase domain mutations, pharmacokinetic and pharmacodynamic factors.

Aims: The purpose of this study was to investigate miRNA-608 role in response to therapy with tyrosine kinase inhibitors (Imatinib). In this study, we analyzed rs9762 SNP located in a miRNA-608 binding site of 3’UTR of BCR-ABL gene and rs4919510 SNP in the mature sequence of miR-608 in CML patients with different response to tyrosine kinase inhibitor therapy. These polymorphisms disrupt the negative effect of miR-608 on its target BCR-ABL. We hypothesized that patients with the age of 15−65 were involved. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol-chloroform method. Genotyping was performed by the PCR-RFLP technique.

Results: Combination of genotypes affecting miR-608/BCR-ABL1 interaction in vitro and in vivo have demonstrated the potency against Philadelphia chromosome (Ph)-positive leukemia cells resistant to ABL TKI as well as in chronic to ABL TKI. We can develop similar method for CML patients. Moreover, patients who carries both SNPs have significant impact on the observed results.

Summary/Conclusions: miRNAs could be a perspective tool for therapy and polymorphisms affecting its regulation should also be considered.

PB1804
TARGETED STRATEGY FOR ABL TYROSINE KINASE INHIBITOR RESISTANT PHILADELPHIA CHROMOSOME POSITIVE LEUKEMIA CELLS
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Background: Although ABL tyrosine kinase inhibitors (TKIs) such as imatinib have demonstrated the potency against Philadelphia chromosome (Ph)-positive leukemia cells, resistance to ABL TKI has been reported in chronic to ABL TKI. We can develop similar method for CML patients. Moreover, patients who carries both SNPs have significant impact on the observed results.

Aims: As leukemia is a genetic disease driven by heritable or somatic mutations, we hypothesized that ABL TKI resistance may often happen due to additional somatic mutations in the oncone.

Methods: We established several TKI-resistant in vitro cell line models. We also investigated model to evaluate the next-generation sequencing (NGS) panel, NGS platform to screen mutational hotspots in 50 leukemia-related genes.

Results: We established ABL TKI resistant cell lines (K562 imatinib-R, K562 nilotinib-R, K562 dasatinib-R, K562 ponatinib-R, Ba/F3 T315I and Ba/F3 ponatinib-R) in this study. We conducted fluorescence in situ hybridization (FISH) analysis on parental K562 and ABL TKI resistant K562 cells. BCR-ABL expression levels were not increased in ABL TKI resistant K562 cells compared to parental K562. We also investigated the intracellular signaling of ABL TKI resistant K562 cells. Phosphorylation of BCR-ABL and Crk-L was reduced in K562 dasatinib-R cells, however, MAPK activation was increased. In K562 ponatinib-R cells, MAPK activity was reduced. We next evaluated the NGS panel (GeneRead DNAseq Targeted Panels V2) to investigate the mutation. We found that several somatic mutations in BCR-ABL were not found in Ba/F3 ponatinib-R cells. K562 ponatinib-R cells were also highly resistant to imatinib, nilotinib and dasatinib. We examined intracellular signaling to determine the effect of phosphorylation of BCR-ABL in parental K562 resistant K562 cells. Phosphorylation of BCR-ABL and Crk-L was reduced in K562 dasatinib-R cells, however, we did not detect additional mutation in K562 nilotinib-R cells. We also investigated the additional somatic mutations in K562 imatinib-R (IDH1 and KRAS), K562 dasatinib-R (IDH1) and K562 ponatinib-R (SF3A1). We could not detect additional mutation in K562 nilotinib-R cells. We next investigated the MEK inhibitor and IDH1 inhibitor activity against K562 imatinib-R and K562 dasatinib-R cells. MEK inhibitor did not induce cell growth inhibition directly. However, combined treatment of ABL TKI resistant K562 with imatinib or dasatinib and MEK inhibitor induced more cytotoxicity than each drug alone. Because activation of PI3K signaling pathway and deregulation of HDAC activity may be a cause of malignant disease in humans, we examined the PI3K and HDAC inhibitor in ABL TKI resistant cells. We found 72 h treatment of oral inhibitor of class I PI3K as well as class I and II HDAC enzymes, CUDC-907 exhibits cell growth inhibition ABL TKI resistant K562 cells and Ba/F3 ponatinib-R cells in a dose dependent manner. In the mouse study, a dose of 20 mg/kg/day p.o of ponatinib and 30 mg/kg/day p.o of CUDC-907 inhibited tumor growth of T315I mutant cells compared with control mice and induced apoptosis in tumor samples.

Summary/Conclusions: Our study indicated that leukemia cells have acquired resistance through somatic mutation or exon 4 deletion in the BCR-ABL. In addition, our findings are suggestive of individual based investigations may be important to evaluate the ABL TKI resistance. We also provide the promising clinical relevance as a candidate drug for treatment of ABL TKI resistant leukemia patients.
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 (I-FISH) during follow-up. The routine dual colour FISH probes are less sensitive to reliably identify der(9) deletions during follow-up. BCR/ABL/ASS1 tri-colour dual fusion (TCDF) probe is highly sensitive and specific in identifying der(9) deletions.

Aims: Our aim was to identify the I-FISH fusion patterns of BCR/ABL/ASS1 TCDF probe and correlate the patterns with patient-specific molecular genetic parameters.

Methods: This was an ethically approved study conducted at a government-funded tertiary care institute. From January 2015 to June 2016, PB I-FISH analysis was performed on European LeukemiaNet defined imatinib-resistant CML patients using BCR/ABL/ASS1 TCDF probe (Abbott Laboratories, Abbott Park, Illinois, USA). The residual BCR-ABL1 transcript load was monitored in international scale (BCR-ABL1FISH) using an automated cartridge-based Genexpert system (Cepheid, Sunnyvale, CA, USA).

Results: On analyzing 37 adult patients, all had residual Philadelphia (Ph) chromosome (100%). Classical 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 illustrates 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 with a loss of residual ABFl on der(9) classical and random signal overlap, respectively. A mean (± S.D) of 29% (± 30) and 18% (± 17) der(9) deleted cells were seen amongst patients with b2a2 and b3a2 BCR-ABL1 transcript types, respectively and this difference was statistically significant (p<0.008). There was also a significant difference in the disease transformation status according to the percentage of der(9) deleted cells (p=0.03). In this regard, patients with progressive disease (accelerated phase/ blast crisis progression) had a mean (± S.D) of 47% (± 35) der(9) deleted cells in comparison to 19% (± 20) such cells in patients without disease transformation. In addition, patients with Ph duplication/triplication had a mean (± S.D) BCR-ABL1FISH levels of 49.478% (± 40.184), in comparison to BCR-ABL1FISH levels of 16.00% (± 19.993) in patients without these anomalies and this difference was also statistically significant (p=0.029).

Summary/Conclusions: Our work would be an appropriate reference material for I-FISH signal interpretation using BCR/ABL/ASS1 TCDF probe. We have demonstrated a high frequency of der(9) deletions, clonal heterogeneity and absence of BCR-ABL1 amplification in an imatinib-resistant Indian CML cohort. For the first time, a significant association of der(9) deleted cell percentage with b2a2 transcript type and disease transformation status has been identified and the same has to be tested in a larger cohort.

PB1806

ARE YOU ACTUALLY SUSPECTING A CHRONIC MYELOID LEUKEMIA WHEN ORDERING A BCR/ABL RT-PCR?

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN). It is characterized by a reciprocal (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 hematimetric parameters, with special focus in basophilia, before performing molecular analysis.

Methods: We retrospectively reviewed 299 BCR-ABL PCR requests received at our laboratory between January 1, 2015 and January 1, 2017. 80% of the total requests were ordered by haematologists physicians, 13.46% by other medical specialties (11.5% internal medicine) and 7.7% from the laboratory. Complete blood cell count (CBC) were analysed by ADVIA 2120. Neutrophilia was defined in our laboratory as absolute neutrophil count of >7.7 x 10^9/L, and basophilia was defined as absolute basophil count of >0.2 x 10^9/L. A total of 299 requests for PCR of BCR-ABL were reviewed by laboratory hematology and oncology performing the classification of Hema
tologic neoplasms criteria. We reviewed clinical history, previous CBC and PBM 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, monocytes, splenomegaly or defined). 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 neoplasia. Our results show that when CML is suspected, basophilia > 0.3 x 10^9/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).

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 x 10^9/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 (PR362FS*21) AND TKI RESISTANCE IN CML PATIENTS FROM RUSSIAN FEDERATION

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Background: Data concerning the impact of BCR-ABL del. c.1086-1270 on TKI resistance in CML is still controversial. This mutation was first described by Curvo et al. (2008) and was thought to confer TKI resistance. However computer modeling performed by Meggyesi N. et al. (2012) revealed disruption of ATP binding site in mutated tyrosine kinase therefore abrogating enzymatic activity. Nevertheless pathogenetic effect of BCR-ABL p.R362fs*21 could be attributed to the formation of heterodimer with “wild type” Bcr-Abl p210 as described by Poulikakos P.I. et al. (2011).

Aims: To assess the impact of BCR-ABL del. c.1086-1270 (p.R362fs*21) on TKI resistance in CML patients from Russian Federation.

Methods: 33 male and 49 female CML patients (age 24-80) with BCR-ABL transcript level >0.1% were included in the study (BCR-ABL del. c.1086-1270 was estimated by nested PCR followed by Sanger sequencing. Initial screening for deletions was performed by means of fragment analysis (Applied Biosystems 3130).

Results: 92 RNA (cDNA) samples isolated from peripheral blood of 82 CML patients were tested. BCR-ABL del. c.1086-1270 (p.R362fs*21) was found in 32 patients (39%). 15 out of 32 (47%) patients with deletion were TKI sensitive while 17 (55%) were TKI resistant. In one TKI resistant case BCR-ABL del. c.1086-1270 was accompanied by BCR-ABL c.844G˃C p.E282Q point mutation not described so far (Figure 1). This mutation was found in BCR-ABL del. c.1086-1270 transcript only and was absent in “wild type” Bcr-Abl p210 transcript amplified from the same patient.

Summary/Conclusions: BCR-ABL del. c.1086-1270 could be found in almost half of CML patients and have no evident impact on the induction of big molecular response in TKI sensitive cases. Our observation that independent c.844G˃C p.E282Q point mutation expressed on the same BCR-ABL transcript with deletion c.1086-1270 (p.R362fs*21) being absent in “wild type” transcript strongly contradicts the hypothesis, that del. c.1086-1270 could be generated by alternative splicing of “wild type” BCR-ABL transcript.

PB1808

PEROXIREDOXIN II ACTIVITY HAS IMPORTANT ROLES TO CONTROL ABL TYROSINE KINASE ACTIVITY IN STIS TREATED CML PATIENTS AND ITS POTENTIAL APPLICATION IN IMATINIB RESISTANCE

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Background: Therapies targeting the redox environment such as over-expression of antioxidant 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 of erythroid progenitor cells. 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 enzymes in leukemia, with limited studies with variable results so far. Altered redox biology in leukemia also has implications for therapeutics.

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 PRXXI 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 Pnx2 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 Pnx2 overexpressed K562 cells. Moreover, Pnx2 overexpressed K562 cells showed further down-regulation of Bcr-Abl oncprotein by IM treatment.

Summary/Conclusions: Our findings may contribute to find a new pathway on which TKIs are working besides the mechanisms of ATP binding competitively, blocking the binding of ABL-kinase and substrate resulting apoptosis of Ph+ cells. In addition we develop the new strategies to overcome the situation of the Imatinib resistant Ph+ 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 granulocyte-macrophage and erythroid hematopoiesis germs in patients bone marrow. Currently we don’t have definitive results regarding tyrosine kinase inhibitors influence on erythropoietic cell characteristics of patients with CML.

Aims: The aim of study was to determine functional characteristics of erythroid progenitor cells of patients with chronic leukemia treated with Imatinib and Nilotinib.

Methods: We studied 300 bone marrow samples from 75 patients: with initial diagnosis of CML (n=7), patients receiving drug imatinib (n=47) and patients who taking nilotinib (n=21). We provide studying of erythroid mononuclears in semisolid in vitro and in vivo cultures. For in 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 minterleykin-6 and interleukin-9. Cultivation was provided 14 days, then counted the number of erythrocyte colonies and provided their morphological studies.

Results: The results showed that the increase of erythroid progenitor cells proliferation rates and the reduction of differentiation rates as a result of the parallel cultivation of patients’ bone marrow cells in vivo and in vitro happen irrespective of the presence of cytokines and growth factors in a normal microenvironment of these cultures. In addition, we showed that bone marrow cells of CML patients form colonies, when placed in the animals’ body without previous allogeneic anemia. Moreover, correlative relationship was found between the number of erythroid colonies and the number of leukemic cells in the patients bone marrow. It was established that the acquisition of 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
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 patients (n=5), CML patients treated with TKIs (n=5) and healthy controls (n=10). We have noticed that PDGF-AA level for newly diagnosed CML patients was higher compared to controls. Interestingly, we have observed a significant difference between the tested groups (p < 0.05). The differences were most pronounced in patients treated with TKIs (both before and during TKI treatment) compared to controls. Notably, we have observed that PDGF levels drop down after TKI treatment, while on the opposite BDNF level in plasma raises with time in CML patients receiving TKIs. We have also monitored these proteins levels over time in the same patients treated with TKIs. This is an interesting finding and requires further investigations to explore any potential correlations between these two proteins.

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 changes in the pathological clone and for monitoring responses to different regimens. Future studies are needed to elucidate the roles of PDGF and BDNF in the pathophysiology of CML.

PB1813

A CASE OF ATYPICAL CHRONIC MYELOID LEUKEMIA WITH LATE DISCOVERY OF JAK2

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Background: Myeloproliferative neoplasms (MPN) include the one hand chronic myeloid leukemia defined by the presence of Philadelphia chromosome and BCR-ABL remodeling, 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 makes no distinction anymore regarding this abnormality, which would include BCR-ABL and V617F JAK2+CM. However, 28 of those cases were described in a 2013 literature review (9). Most patients developed either a V617F JAK2 mutation during treatment by tyrosine kinase for a BCR-ABL+CML, a BCR-ABL+CM for 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 a 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 then, the patient was on nilotinib treatment for chronic myeloid leukemia (CML) at moderate dose (600mg daily) followed then by polycythemia (Hb: 16.7–19 g/dL) that were first attributed to hemocentromast and inflammation due to recurring 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 fever. A physical examination revealed multiple skin lesions with irregular shape and heterogeneous coloration. A biopsy of a lesion was performed and revealed a variant of angio-kerato-syringoma that were first attributed to hyperkeratosis and inflammation. The skin lesions were found to be resistant to various local treatments (hormonal), but cleared after the administration of topical corticosteroids. A skin biopsy revealed the presence of abnormal melanocytes. A skin biopsy revealed the presence of abnormal melanocytes. A skin biopsy revealed the presence of abnormal melanocytes. A skin biopsy revealed the presence of abnormal melanocytes. A skin biopsy revealed the presence of abnormal melanocytes.

PB1811

Abstract withdrawn.
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 (61%) patients lost DMR. Median time off-therapy for these patients was 3 months (2-8), and only 1 lost DMR after 6 months. Therapy was restarted in all 13 patients (2 in MR1, 4 in MR2, 7 in MR3), 10 achieved a second DMR after a median interval of 2 months (1-7); 2/13 patients are in M3 after 7 and 12 months, 1 patient is not yet evaluable. Ten out of 11 patients treated with INF before imatinib remained in TFR. Of note, the type of bcr-abl transcript was significantly linked to DMR loss: after TKI discontinuation, 32/40 e14a2 patients (80%) maintained DMR vs 4/9 e13a2 patients (44%) (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 of the sustained DMR is significantly higher as compared with e13a2 patients. Moreover, after discontinuation, e14a2 have significantly lower probability of DMR loss than e13a2. These data confirm that e14a2 is associated with a more favorable CML disease profile than e13a2 (Jain et al., Blood 2016); in addition they show that e14a2 is a favorable prognostic factor for TFR maintenance.

PB1815

COMPARATIVE ANALYSES OF NILOTINIB VS HIGH-DOSE IMATINIB VS SUSTAINING STANDARD-DOSE IMATINIB IN PATIENTS WITH CP CHRONIC MYELOID LEUKEMIA FOLLOWING SUBOPTIMAL 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, up to 70%-75% of CML patients achieve undetectable molecular response at specific time points, achieving major molecular response at 12 months showed minimal event-free survival (EFS) benefit, compared with not achieving MMR but having complete cytogenetic response (CCyR). In addition, the best treatment for these patients remains less clear.

Aims: In this study, we investigated the efficacy of nilotinib (NIL) versus high-dose IM versus sustaining standard-dose IM for the patients with CCyR with sub-optimal molecular response to first-line IM therapy.

Methods: Early CP CML patients who have achieved CCyR but not MMR after 18 months on first-line IM therapy at a daily dose of 400 mg were divided into three treatment groups; nilotinib (NIL 400mg BID (800mg/day; group 1) vs IM 400 mg BID (800mg/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.

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 enrolment was 36 months (range, 1-36), 45 months (range, 21-63), and 36 months (range, 21-63) for each group, respectively. All patients in group 1 remained NIL treatment, 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), intolerance (n=2) and 2 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 different in group 1 vs 2 (P=0.195) and group 2 vs 3 (P=0.297). CI of MRY by 36 months showed a trend of higher in group 1 than the other two group (11.7% vs 0% vs 2.0%, 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 CHRONIC MYELOID LEUKEMIA 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 hospitals of 77 Russian cities having been observed during the period from 2006 to 2016. **BCR-ABL1** kinase domain point mutations in mRNA samples from peripheral blood cells were analyzed by means of PCR followed by Sanger sequencing. Statistical analysis was performed using SPSS 22.0 (IBM, USA) and Excel 2013 (Microsoft, USA). Critical p-value was set to 0.05.

**Results:** 1077 TKI resistant CML patients were analyzed, among them were 41.5% men (n=447) and 58.5% women (n=630), median age – 50 (from 15 to 74). **BCR-ABL1** mutations were found in 30.8% (332/1077) CML pts. We have detected a total of 415 mutations in 332 patients, giving rise for 58 different mutation variants. Mutation associated resistance rate was higher in women compared to men, being 62% in women vs 41% in men. IM associated resistance rate was statistically lower in both sexes (IM H396R and H399R mutations were statistically more frequent in women, meanwhile T315I mutation prevailed in men (Pearson’s χ²=0.05)). It was of a sudden that **BCR-ABL1** mutation distribution significantly varied according the particular CML pts city location throughout the different regions of Russia. Although for the period from 2006 to 2016 there were no detectable changes in mutation frequency spectrum (Pearson’s χ² is 0.062), the total amount of mutations associated with **TKI** CML resistance has decreased from 36.6% in 2006-2008 to 24.96% in 2013-2016, but still remained significant. For particular mutations the following dynamics was detected: 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 far as different **BCR-ABL1** kinase domain mutations are associated with various types of mutation associated resistance to **TKI** treatment, the detection of trends in mutation distribution in CML patients receiving **TKI** treatment is very important for long time treatment strategy decision making, and for the regional analysis of resistance. We believe here that 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**

**IMATINIB (IM) 400MG IN PATIENTS WITH CML1ST CP RESULTS IN A MORE HIGHER MOLECULAR RESPONSE RATE AT 6 MONTHS COMPARED TO IM/ HYDROXYUREA. FINAL RESULTS OF THE CML2004 STUDY.**

**NTC 02480608**

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**Background:** Imatinib (IM) monotherapy remains an acceptable option to treat **CML** in patients with chronic myeloid leukemia (CML) in the chronic phase (CP). Hydroxyurea (HU) is effective in controlling elevated white cell counts and has been widely used to treat CML prior to the era of tyrosine kinase inhibitors (TKIs). The combinations of IM and HU have been tested in vitro and showed a additive suppression of CML CFU-GM cells. Combinations of IFN and HU and cytoreductive HU and IFN have been tested in vivo, but no data are available for the combination of IM and HU in CML.

**Aims:** The East German Study Group conducted a phase I study to identify the dose of **IM** in combination with standard dose **IM** (400mg daily) that would result in mild myelosuppression (white blood cell count 3,000-4,000/mL). Starting dose of **IM** was increased in steps to achieve myelosuppression of the patients to a maximum of 3,000 mg daily. According to protocol, 500 mg **HU** was identified as the starting dose for the randomized phase II study which tested the combination vs standard dose **IM**, with the rate of major molecular response (MMR) at 18 months as the primary end-point.

**Results:** Starting in 2002, 20 adult patients with 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, NCT01533591) was performed. The initial treatment was NIL 300 mg Bid or NIL 400 mg Bid. Definitions: major molecular response (MMR), BCR-ABL1** ratio < 0.1%, deep molecular response (MRD); BCR-ABL1** ratio < 0.01% 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 (e1a2 or e1b2a2) n=7) and patients with unknown transcript type (n=10) were excluded: 328 patients were evaluable, 38% with e1a2 transcript, 53% with e1b2a2 transcript and 9% 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 65 months (range 24-82 months). The response rates and the survival probabilities were compared in patients with e1a2 transcript and patients with e1a2 transcript (N=174), but the differences were not significant: MMR by 12 months, 66% vs 72%, p=0.244; MR4.0 by 36 months, 56% vs 66%, p=0.067; estimated cumulative incidence of MMR, 82% vs 88%, p=0.135; estimated cumulative incidence of MR4.0, 60% vs 69%, p=0.101; estimated FFS, 88% vs 93%, p=0.547; estimated OS, 98% vs 94%, p=0.547. The responses and the survival probabilities of patients co-expressing the e13a2 and the e1a2 transcripts (N=30) were similar to or even better than the ones of e1a2 patients. Grouping together the patients with e1a2 transcript alone and the patients with co-expression of both transcripts (N=174+30=204), and comparing them with patients with e1b2a2 transcript alone (N=50), it was clear that the e1a2 transcript was more frequent in women, meanwhile the e1b2a2 transcript was statistically more frequent in men: 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 in the intention-to-treat analysis. According to the study protocol, patients from the CML IV study were to be added to obtain equal numbers for analysis. To arrive at a total of 77 IM patients, from study IV another 49 patients were selected by propensity score matching. The median age of the 154 patients was 55 years (range 18 – 82). The ELTS prognostic score was suitable 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<not significant). Primary endpoint was MMR rate at 18 months. There was no significant difference between IM/HU (65.8%) and IM (66.0%). At 6 months, MMR rate in the (CTCAE) vs 41.1% (P=0.0382) 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 OS was 500mg (range 152-3000); the median IM dose was 400 mg (range 145-612mg). The majority of adverse events in general or of adverse events of grade 4 were not different between the two arms, but cumulative incidences showed an earlier occurrence in the IM/HU than in the IM arm (p=0.0343, Gray test)

Summary/Conclusions: Compared to Imatinib only, the combination of Imatinib and HU results in a lower MMR rate at 6 months but a similar MMR rate at 18 months. Furthermore, IM/HU was associated with more early adverse events. There was no indication of a beneficial effect in the treatment of CML patients in 1st chronic phase using the combination of IM with HU.

PB1820 A MULTICENTER, OBSERVATIONAL, AMBISPECTIVE STUDY EVALUATING EFFICACY AND SAFETY OF GENERIC IMATINIB COMPARED TO GLEEVEC IN CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE - 3 MONTHS RESPONSE ANALYSIS K. Pagnano1, E. Miranda1, N. Clemetinio2, G. Magalhaes2, A. Coelho2, E. de la Guardia3, J. Bortolini8, C. Pinna9, M. D. L. Chauffaille10, R. Centrone11, C. De Souza12

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Background: The efficacy of branded imatinib (Gleevec) in the first-line treatment of chronic myeloid leukemia (CML) has been demonstrated in several clinical studies. However, there are few consistent data in the literature on the efficacy and adverse effects of generic formulations of imatinib. In Brazil, CML patients have been treated in the national public health system with generic imatinib since June 2013.

Aims: The present study aims to evaluate the efficacy and safety of generic imatinib in the treatment of CML in comparison with the reference drug (Gleevec) in the first three months of imatinib treatment.

Methods: This is a multicenter, observational, ambispective, cohort-type study. The study was initiated in January 2015 with the intention participation of 17 Brazilian centers. In the prospective group, were selected chronic phase CML patients who started their first-line treatment with generic imatinib between January 2015 and October 2016, whereas retrospective group was treated with branded imatinib between January 2010 and December 2011. All patients started imatinib less than six months from diagnosis. Study data were collected and managed using REDCap electronic data capture tools. Demographic data were collected at diagnosis: age, gender, Sokal, Hasford, EUTOS score, comorbidities, cytogenetics, BCR-ABL transcript type. The definition of the responses followed the CML IV study protocol. Complete response (CR) was defined as 42%-52%; intermediate risk 42%-31% and high risk 45%-67% (P=0.48). There was no difference between the groups concerning gender, Hasford, EUTOS scores, ECOG, blood cell counts at diagnosis and before starting imatinib and BCR-ABL transcripts. Responding rates, there was no differences between the hematological complete cytogenetic responses and rate of BCR-ABL transcripts>10% at three months. However, there was a higher rate of failure at three months according to the ELN 2013 criteria in the prospective group (14.9% versus 4.7% Gleevec group, P=0.04). There was no significant difference in grade 3 and 4 hematological and non-hematological toxicity, but there was one early death in the prospective group (acute peripheral arterial occlusion and renal failure). Four patients discontinued imatinib: one from Gleevec group (resistance) and three from the generic group due to intolerance (1) and resistance (2).

Summary/Conclusions: According to ELN-2013 criteria, there was a higher rate of failure at three months but no difference in toxicity. The register is ongoing; the confirmation of this data and the impact in prognosis will be evaluated in the long-term follow-up, after increasing the number of patients.

PB1821 COMPLEX ADDITIONAL CHROMOSOMAL ABERRATIONS IN PH-POSITIVE CELLS IMPACT ON CHRONIC MYELOID LEUKEMIA PATIENTS’ SURVIVAL IN THE ERA OF TYROSINE KINASE INHIBITORS M. Fominykh1, O. Shuhov2, I. Martynkevich2, V. Shuvav2, E. Chelysheva2, A. Turinka2

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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 considering 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 (CP) patients at diagnosis was determined in 23 (77%) patients. Imatinib was used as first-line in 20 (67%) patients, 4 patients from this group had «major-route» ACA. Accelerated phase was defined in 7 (23%) patients. In that group treatment of 8 patients was started with Imatinib and Dasatinib was given initially for one patient. «Major-route» ACAs (trisomy 8, 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.
PB1822
BCR/ABL1 TRANSLOCATION 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 transscripts can be produced during chromosomal translocation, which lead to formation of the BCR/ABL fusion gene in patients with chronic myeloid leukemia (CML). Previous results of a few large studies 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 transcriptions 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 same 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), event-free survival (EFS), and event-free survival (EFS) were calculated using Kaplan-Meier method. Event in EFS was defined as death of a patient or progression of disease, or loss of CCR or MMR.

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. Survival probability and the proportion of patients with advanced chromosomal abnormalities in Ph-positive cells. No correlation of transcript type with age or sex was observed. Transcript e13a2 was associated with higher WBC (120x10^9/L vs 95.3x10^9/L, P=0.02) and lower baseline percentage of eosinophils (p=0.041). No differences were found in other differential counts of peripheral blood, hematological response (2nd 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.

PB1823
ANALYSIS OF GENERIC IMATINIB EFFICACY IN CHRONIC MYELOID LEUKEMIA TREATMENT: MORE THAN FOUR YEARS OF EXPERIENCE IN SOUTHERN SERBIA
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Background: Tyrosine kinase inhibitors (TKIs) are the golden standard in the treatment of chronic phase chronic myeloid leukemia (CP-CML) due to their high efficacy and mild toxicity profile. Because of the high price of these drugs, the use of generics is encouraged to reduce health care costs. In the literature, there is still limited data and some concerns about the effectiveness of generic imatinib (GI), although there is a growing number of countries in which it is used instead of branded imatinib (OI).

Aims: The objective of this study was to evaluate efficacy and safety of GI in newly diagnosed CP-CML patients treated with frontline GI and in patients switched from OI to GI.

Methods: Cohort of 101 adult patients with CP-CML was analysed, treated with GI from August 2012 to February 2017. First group consisted of 53 patients treated with GI (Anzovip). According to European LeukemiaNet (ELN) 2013 guidelines, rate of optimum therapeutic response was analysed, as well as rate of treatment failure at 6 and 12 months.

The second group consisted of 48 patients switched from GI to GI, in which the rate of achieved therapeutic response at the time of switching and the rate of maintenance of CCyR, MMR and MR4 after a minimum of 12 months under therapy with GI were both analysed. In order to investigate safety of GI, in both groups rate of hematological and non-hematological adverse effects (AEs), all grades according CTCAE criteria, were analysed.

Results: Analysis of the response by ELN in the group with GI showed that at 6 months 33/53 (62.3%) patients achieved CCyR, BCR-ABL<1% was in 27/52 (51.9%) patients, while 15/52 (28.8%) of patients achieved MMR. At 12 months of therapy, 35/49 (71.4%) of analysed patients achieved CCyR, and 25/49 (48.9%) achieved MMR. ELN criteria for treatment failure at 6 months was 12/52 (23.1%) patients, while at 12 months ELN criteria satisfied 13/49 (26.5%) of analysed patients. After 18 months of therapy with GI the rate of CCyR was 35/46 (76.1%) and MMR was 28/45 (62.1%) and showed trend of increase. During the median follow-up period of 23.8 months 3 patients have progressed to blast phase and total of 7 patients died. In the second group, in time of switching from OI to GI, the rates of achieved CCyR, MMR and MR4 were 82.5%, 65.8% and 49% of patients respectively. The rate of maintenance of previously achieved CCyR was 95%, of MMR 88% and of MR4 72% in the course of the median duration of GI exposure of 37.8 months. When comparing first and second group respectively, the rates of patients which have been switched to 2nd generation of TKI because of the failure or intolerance to imatinib were 27.8% vs 24.8%, and 60.5% vs 64.5% of them achieved secondary optimal therapeutic response (CCyR plus MMR), while 25% vs 20% of them have been sent to BMT. Group switched from GI to GI had not significantly different rates of acquired biological AEs compared to GI group (12.7% vs 24.2%, p=0.991). Furthermore, the rate of grade 3-4 hematological AEs were similar in both groups (13% vs 15%, p=0.952).

Summary/Conclusions: Results of this study with extended follow-up of more than four years are further evidence of that the generic imatinib is at least non-inferior to the original imatinib regarding efficacy both when used initially or as a subsequent replacement for branded imatinib.

PB1824
ACHIEVING OPTIMAL RESPONSE AT 12 MONTHS IS ASSOCIATED WITH A BETTER HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A PROSPECTIVE, LONGITUDINAL, SINGLE CENTER STUDY
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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 based on EQ-5D-5L scores were measured for patients at diagnosis and 6 and 12 months of therapy and post-treatment HRQoL 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 (warning or failure), and overall survival rate at 5 years was significantly higher in those who achieved optimal response at 12 months (P =0.047). Achieving optimal response at 12 months was associated with better role limitations due to physical health problems (P =0.019) and role limitations due to emotional problems (P =0.0160) and was the sole factor associated with significantly improving physical component summary over time (P =0.0160). In addition, achieving optimal response at 6 months had a tendency of high physical functioning (P =0.0674), social functioning (P =0.0571), and role limitations due to emotional problems (P =0.069). Females had significantly higher scores than males in pain (P =0.0471) and physical functioning (P =0.0471). Age >65 years 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 frontline tyrosine kinase inhibitor.

PB1825
MULTI-COUNTRY RETROSPECTIVE CHART AUDIT STUDY TO EXAMINE DEEP MOLECULAR RESPONSE (MR4.5) ASSOCIATED WITH GI IN CP-CML PATIENTS TREATED IN CHRONIC PHASE
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Background: European LeukemiaNet (ELN) 2013 guidelines, rate of optimum therapeutic response was analysed, as well as rate of treatment failure at 6 and 12 months.
 seabased 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 (AFT) 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.

PB1827

A NATIONWIDE OBSERVATIONAL STUDY OF PONATINIB IN CHRONIC MYELOCYTIC LEUKEMIA OUTSIDE CLINICAL TRIALS

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Methods:

The Kaplan Meier estimator; unadjusted HR=1.09, 95% CI [0.87, 1.38], p=0.46); adjusting 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, mean age of 57 years, and were 35% female. 8% of 2L nilotinib and 22% of 2L dasatinib pts were treated with the other 2nd generation TKI (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) as 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 year 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 [0.42, 0.86], p<0.01) were inversely associated with achieving MR4.5.

There was no significant difference in MMR achievement between 2L nilotinib and 2L 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 [0.42, 0.86], p<0.01) were inversely associated with achieving MR4.5.
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=6, 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 in 60% (N=32) of patients and in 24% of them (N=9) the dose was reduced during treatment. The median survival time of patients with ponatinib was 38 months (95%CI: 30 to 47 months) (Figure 1). Patients died because of cerebrovascular event (N=1), sepsis (N=2) or graft vs host disease that developed shortly after HSCT (N=1). *Response assessment:* Response assessment as available for 32 patients (86%). Seventy percent (N=22) achieved molecular response, of which 60% (N=13) achieved at least major molecular response. The median time to maximal response was 7 months (range: 3 to 28 months). *Drug discontinuation:* Twenty four percent (N=9) discontinued ponatinib after a median of 7 months (range: 1 to 18 months) because of disease progression (N=6) or severe adverse effects in two patients (cerebrovascular event and severe pancytenia).

**PB1828**

**MOLECULAR RESPONSE TO THERAPY WITH TYROSINE KINASE INHIBITORS IN PATIENTS WITH BCR-ABL1(C) CHRONIC MYELOID LEUKEMIA PRESENTING WITH AN ISOLATED THROMBOCYTOSIS AT THE ONSET**

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**Background:** Detection of BCR-ABL1 e13a2 or e14a2 transcripts (major break- points) or BCR-ABL1 fusion transcripts (translocations, also known as the Philadelphia chromsome) 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 calls a molecular reduction (MR) of ≥4.5 logs below baseline (i.e. MR4.5 or 0.0032%IS).

**Methods:** To clinically validate the QuantiDx qPCR BCR-ABL1 IS Kit and to reifirm the clinical utility of BCR-ABL1 RT-qPCR monitoring in patients with (9;22) positive CML, a correlation between molecular response (MR) values and long-term outcome was determined.

**Results:** Of the 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 mutarion, 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 MR3.0). Three laboratories performed BCR-ABL1 testing on banked RNA specimens from 96 chronic phase CML patients from 2 hospitals drawed 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 mutarion, 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.

**Conclusions:** Of the 96 patients had MR>3.0 at 12-18 months post-TKI. Of these 96 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<3.0 at 12-18 months post-TKI. Of these 45 patients 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 MR<3 vs 80% (95% CI 68%-93%) for MR≥3].

**PB1829**

**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- points) or BCR-ABL1 fusion transcripts (translocations, also known as the Philadelphia chromsome) 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 calls a molecular reduction (MR) of ≥4.5 logs below baseline (i.e. MR4.5 or 0.0032%IS).

**Methods:** To clinically validate the QuantiDx qPCR BCR-ABL1 IS Kit and to reifirm the clinical utility of BCR-ABL1 RT-qPCR monitoring in patients with (9;22) positive CML, a correlation between molecular response (MR) values and long-term outcome was determined.

**Results:** Of the 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 mutarion, 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 MR3.0). Three laboratories performed BCR-ABL1 testing on banked RNA specimens from 96 chronic phase CML patients from 2 hospitals drawed 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 mutarion, 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.

**Conclusions:** Of the 96 patients had MR>3.0 at 12-18 months post-TKI. Of these 96 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<3.0 at 12-18 months post-TKI. Of these 45 patients 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 MR<3 vs 80% (95% CI 68%-93%) for MR≥3]. Specimens with MR values ranging from MR1 to MR4 showed an average%CV of 2.8%. Day to day agreement was high with MR SD by operator from 0.000 to 0.080. Site to site agreement was high with MR SD by site ranging from 0.000 to 0.069. The 95% LOD for both transcripts (e13a2 & e14a2) was MR4.7 (0.002%IS), allowing sensitive detection of the MR4.5 cutoff that defines “complete molecular response” in ongoing treat- ment.

**Acknowledgements:** Partial support by the National Science Fund.
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 the best way of assessing response. 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.

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 and 6 months might guide the decision to switch TKI, but patient’s comorbidities, possibility of discontinuation and cost of therapy should also be considered.

PB1831

PREDICTIVE PARAMETERS FOR IMATINIB FAILURE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Development of tyrosine kinase inhibitors (TKIs) has significantly changed natural course of chronic myeloid leukemia (CML) and increased 10 year overall survival from 10-20% to 80-90%. Until recently, imatinib was the standard first-line treatment in CML. In 2013, nilotinib and dasatinib were approved as alternative front-line options. However, none of three TKI has been shown to have a clear survival advantage so this raised a debate on treatment selection. The early identification of patients expecting poor outcome is crucial for offering an alternative TKI regimen.

Aims: to analyze predictive parameters for Imatinib response as first-line treatment of CML patients.

Methods: The study was conducted on 168 consecutive patients with chronic phase of Ph+ CML who were diagnosed and treated at single university hospital from December 2000-January 2015. Following data were analyzed in terms of response treatment to Imatinib: demographic characteristics; currently used prognostic scores (Sokal, Hasford, EUTOS); liver and spleen size; laboratory parameters; influence of comorbidities analyzed by three scores (ACE 27, HCI-Cl, SCIRS); occurrence of second malignancies; conventional cytogenetic test, type of therapy, duration of therapy, cytogenetic responses, overall survival (OS) and outcome.

Results: The mean age at diagnosis was 48±14.4 years (range: 18-74) with 87.5% of patients>65 years. The OS at 5 and 10 years was 97% and 91% respectively. Overall response to Imatinib treatment was as the follows: 131 patients (78.5%) achieved MMR (83.3% major molecular response), 16 patients (9.5%) had no cytogenetic response, 2 patients (1.2%) had hepatic toxicity verified by liver biopsy in the first six months of Imatinib treatment and 1 patient (0.6%) was lost from follow-up. After achievement of MMR, 25 patients (19%) had a progression of disease by losing MMR or the achievement of CCyR, 25 patients (19%) had a progression of disease by losing CCyR or the achievement of AP/BP. Median time to progression was 24 months (range 12-102). After the median follow up of 87 months in 61 patients (36.3%), the Imatinib failure was verified. All three prognostic scores (Sokal, Hasford, EUTOS), age, gender, hemoglobin level, leucocyte and platelet count, splenomegaly, eosinophilis and basophilis in peripheral blood were not found to be statistically significant for the Imatinib failure. Cox regression analysis identified hepatomegaly (p=0.001), leukocytosis 100x109/L (p=0.001), blood blasts>1% (p=0.002) and presence of additional cytogenetic aberrations (ACA) (p=0.002) as a predictors of Imatinib failure. Accordingly, we assigned risk scores based on hazard ratios (HR) to hepatomegaly (HR=4.089; 2 points), leukocytosis ≥100x109/L (HR=3.973; 95% CI for HR 2.237-7.053, p<0.001), presence of additional ACA (HR=11.110; 2 points). A final 3-tiered prognostic model named IMA-FAIL was thus developed, as low (score 0), intermediate (score 1-3), and high risk (score ≥4), according to which imatinib failure had 17% (8/47) of patients in low, 34.9% (30/88) in intermediate and 76.7% (23/30) in high risk group (HR=3.973, 95% CI for HR 2.237-7.053, p<0.001). In addition, presence of comorbidities as well occurrence of secondary malignancy were not predictors for imatinib failure.

Summary/Conclusions: Hematologists are facing with challenge of making decision which TKI to choose upfront with increasing a chance to achieve best possible response. The new score allows better selection of patients who are suitable for treatment with Imatinib and may guide the clinical decision for front-line treatment of CML.

PB1832

A MULTICENTRE AUDIT OF SYMPTOMS AND QUALITY OF LIFE IN IRELAND CML PATIENTS ON TYROSINE KINASE INHIBITORS

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Background: The development of tyrosine kinase inhibitors (TKIs) over the last 20 years has dramatically improved the outcomes for patients with every stage of chronic myeloid leukaemia (CML). Since the approval of the first TKI, imatinib, in 2001, there are now currently 5 oral TKIs available. Three are approved for frontline use (imatinib, dasatinib and nilotinib) and 2 others (bosutinib and ponatinib) approved for intolerance or failure of prior TKI. Because CML patients need to continue TKI treatment indefinitely, 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 across multiple centres in Ireland, using the MD Anderson Symptom Inventory (MDASI) tool.

Methods: Across 7 centres in Ireland, a total of 87 CML patients currently on TKIs were identified. The mean age was 60yrs with an equal sex distribution (44 male, 43 female). All of these patients were in chronic phase. 79% of patients were in MMR (major molecular remission) at the time of survey. 53 patients were on imatinib, 19 patients on nilotinib, 13 on dasatinib and 2 on bosutinib. Patients from the 7 centres were surveyed at varying time periods between July 2015 and Feb 2017. Patients were contacted by phone. Symptom burden and QOL were assessed using the MD Anderson Symptom Inventory. Because CML patients received TKI validated tool, the standard clinical assessment of symptoms, as well as 6 interference items. The questionnaire took on average 5min to complete and asked patients to rate their symptoms on a scale of 1-10 as experienced over the preceding 24 hours.
Results: Of the 87 patients surveyed, the most commonly prevailing symptoms were 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 have different frequency of symptoms and with others. Patients taking second generation TKIs did not appear to have any difference in frequency or severity of symptoms or in QOL compared to patients on imatinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential 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 factors was used. The criterion of efficacy was the MMR. The considered costs were: the cost of the TPE program, costs in terms of use of care.

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 have different frequency of symptoms and with others. Patients taking second generation TKIs did not appear to have any difference in frequency or severity of symptoms or in QOL compared to patients on imatinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential 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.

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: 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 with other drugs other 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 (15-76) (38 years in HSCT-group), 2 pts were in BC at the time of HSCT, 5 pts were in AP, 7 pts were in CP22. The number of points on EBTM scale: 3-4 points –1275%) pts, 5-7 points –4(25%) pts, 11 (69%) pts received more than 2 lines TKI treatment. In allo-HSCT group 11 (69%) pts had unrelated donors. Conditioning regimen in 13 (81%) pts had reduced intensity, in 3(19%) pts had MAC. Me time to HSCT after T315I detection was 10 months (1-38). Mutation analysis was performed by Sanger sequencing. Overall survival (OS) was estimated by Kaplan-Meier method with log-rank test for comparison between groups. Cox regression was used for multivariate survival analysis that included next covariates: age, phase on the time of mutation detection, performance of allo-HSCT, time to T315I detection from TKI start.

Results: Me 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, 2 in HSCT group and 3 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 investigat ed 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 22.
Methods: This cross-sectional study comprised 85 patients with CML in chronic phase, treated with imatinib and nilotinib, at the Clinical Center of Vojvodina, Serbia. Thyroid function was assessed by analyzing the serum FT3, FT4 and TSH levels. Hypothyroidism in relation to TKI therapy was defined as newly diagnosed hypothyroidism (while the patient was already on TKI therapy) requiring hormone substitution therapy or serum FT4 level <11.5 pmol/l and/or FT3 level <2.45 pmol/l. Patients with previous medical history of thyroid dysfunction were excluded. The duration of TKI treatment varied from 2 month to 10 years. The dose of imatinib was 400mg daily, while nilotinib was dosed 800mg a day.

Results: From the total number of patients included, 37 (43.53%) were female and 48 (56.47%) male. Age range was 21-84. The prevalence of hypothyroidism (clinical, and subclinical) was 8.23% (n=7) which is in accordance with the prevalence in general population. Three patients (3.53%) were diagnosed to have subclinical hypothyroidism (defined as normal serum FT4 and TSH >5.50 mU/L). Hypothyroidism was more common in males (71.5%, p<0.50, not statistically significant). In patients treated with imatinib, 2 (3.4%) had subclinical, while 3 (5.01%) had clinical hypothyroidism. Of the 26 patients treated with nilotinib, subclinical hypothyroidism was detected in 1 (3.85%), as well as clinical hypothyroidism (3.85%). Other thyroid dysfunctions were not detected.

Summary/Conclusions: Hypothyroidism was the only thyroid dysfunction in our study, and the prevalence of hypothyroidism in our study did not differ from general population. Additional study on a larger sample size and evaluation of antibodies is required.

PB1836 RESPONSE RATES AND SURVIVAL OF PATIENTS WITH CHRONIC MYELOID LEUKAEMIA (CML) TREATED WITH IMATINIB: 11 YEAR EXPERIENCE OF A TEACHING HOSPITAL M. Ruparelia1,*, P. George1, D. Brownings1, K. Marshall1, A. Hunter1, M. Martin1, K. Hodgson1 1University Hospitals Leicester, Leicester, United Kingdom

Background: In large trials, patients with chronic myeloid leukaemia (CML) treated with Tyrosine Kinase Inhibitors (TKIs) have 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 age-related normal 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.

Methods: A retrospective case record review was undertaken on CML patients identified from the regional cytogenetics department. Imatinib was available for routine prescription in the UK from 2003, so a 11-year period from 2003 to 2014 was selected to allow for adequate follow-up.

Results: In total 83 patients were newly diagnosed in this time period. Four patients, treated on SPRIT2 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 13-93) with 50% being male. The median follow up was 73 months (range in living patients 29-163 months). Fifteen patients have died (19%). The median age of diagnosis of these was 73 years. Two deaths were transplant-related, both in patients who had failed available TKIs and had mismatched transplants. The only treated patient who died of accelerated disease was intolant to 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 successful mast cell leukemia transplant. The median time to CCyR was 16 months (range in patients 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 with limited life expectancy who have taken a pragmatic approach; three are rejected 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.

Summary/Conclusions: This data shows the real life experience of patients treated for CML 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. Using an intention to treat analysis in this unselected population, up front imatinib with appropriate escalation of treatment where response is unsatisfactory achieves an MMR rate of 76%. This offers reassurance that where appropriate monitoring is feasible, imatinib remains a good first choice for patients presenting with CML.

PB1837 FRONT-LINE NILOTINIB IS A BETTER CHOICE THAN FRONT-LINE IMATINIB FOR CML PATIENTS WITH DELAYED TREATMENT: 11 YEAR FOLLOW-UP I. Cojbasic1,2,*, L. Macukanovic Golubovic1,2, M. Vucic1,2, N. Govedarovic1,2 1Clinical pathology, cytology, and human genetics, Clinical Center of the University of Sarajevo, 2Faculty of Natural Sciences, University of Sarajevo, 3Hematology, Clinical Center of the University of Sarajevo, 4Genetics, University of Sarajevo, 5Hematology, Clinical Center Zenica, Sarajevo, 6Hematology, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

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 front-line imatinib and front-line 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 multicentre retrospective cohort study. Patients who waited less and more than 6 months from the start of therapy. Nilotinib became available as front or second-line therapy in March 2011. Standard patients’ variables were collected and disease progression was established as loss of CCyR or MMR. Survival was estimated using the Kaplan-Meier method and compared using the log-rank test.

Results: We analyzed 149 patients (median age was 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) in patients who waited less and more than 6 months was 0 months (range 0-6) vs 15 months in the waiting group (range 9-63). At 11 years, overall survival for patients on front-line imatinib (Group 1) and front-line 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 studied groups (20/118 vs 4/31). When we analysed delayed treatment 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 line delayed nilotinib achieved 83% vs 77% for CCyR and 78% vs 69% for MMR, respectively.

Summary/Conclusions: Our results after 11 years of follow up suggest that nilotinib demonstrated improved efficacy over imatinib therapy. Achievement of CCyR and MMR at 24 months was higher in patients on front-line 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 FRONTLINE IMATINIB I. Cjojasic1,*, M. Macukanovic Golubovic1,2, M. Vucic1,2, N. Govedarovic1,2 1Clinical pathology, cytology, and human genetics, Clinical Center of the University Clinical Hospital Mostar, Mostar, Bosnia, Nis, Serbia

Background: The tyrosine kinase inhibitor (TKI) imatinib was the first targeted therapy for patients with chronic-phase chronic myeloid leukemia (CP-CML), and its introduction has had drastic consequences on patient outcomes including response and survival compared to previous therapies. Earlier studies have indicated that the effect of age at diagnosis of CP-CML was minimized in patients treated with imatinib: fewer responses but the same outcome for older patients. However, recently published results from clinical controlled trials indicated that there were differences in clinical outcome depending on age at diagnosis of CP-CML.

Aims: The aim of this study was to evaluate impact of age on the treatment outcome in patients with chronic myeloid leukemia treated with frontline imatinib.

Methods: A newly diagnosed CP-CML patients treated and followed in our institution were surveyed retrospectively from August 2006 to August 2016. To this age, cohort was divided into three groups: young adults (18-45 years) (YA), middle aged adults (46-64 years) (MA) and elderly persons (65 and more years) (EP). Patients’ demographics, disease risk scores, duration of imatinib therapy and follow-up, cytogenetic and molecular responses,
Enzymopathies, membranopathies and other anemias

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/13/14/1330; 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, xenogeneic 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 ektacymetry has become a powerful procedure to measure red cell membrane deformability and therefore for the diagnosis of red blood cell membrane disorders.

Aims: The aim of this study is to evaluate osmotic gradient ektacymetry as an adequate assay to perform screening of membranopathies, focusing on the differential diagnosis between HS and non-spherocytic membrane defects such as HE and dHSt.

Methods: A total of 75 patients with chronic hemolytic anemia oriented as hereditary RBC membrane disorders (hemoglobin disorders discarded and negative Coombs test) were included during a period comprised between January 2015 and August 2016. Normal controls were obtained from blood donors. Osmotic gradient ektacymetry was performed using the osmocsmac module of the Laser-assisted Optical Rotational Deformability Cell Analyzer: LoRRCa MaxSis (RR Mechatronics). Evaluation of osmocsmac 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 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.

PB1839

CHARACTERIZATION OF HEMATOPOIETIC SAMPLES FROM PYRUVATE KINASE DEFICIENCY PATIENTS

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Background: Pyruvate Kinase Deficiency (PKD) is an autosomally 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/13/14/1330; 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: 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, xenogeneic 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.
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 osmolar 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 Ohyper and a slight increase in Elmin. Reference ranges for each osmolar parameter were established with 171 healthy subjects and compared with the values of the parameters obtained from the different RBC membrane disorders. ROC curve analysis was performed for HS and each one of the non-HS groups separately. The results determined that EImax 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 Elmin cut-off to differentiate HS hemolysis not in just in genetic counseling and future antenatal diagnosis (sensitivity 98.40%, specificity 99.42%), while the optimal Omin cut-off to differentiate HS from HE was >159.0 (sensitivity 95.38%, specificity 85.71%). Expressing the results as% of variation in relation to the mean of our normal controls, the best combination of parameters for HS diagnosis would be Elmin <3% and Omin >5.2%. This combination of cut-off values for EImax and Omin (sensitivity 98.40%, specificity 99.42%) and Omin (sensitivity 95.38%, specificity 85.71%) 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 no-HS (sensibility 93.85%).

Summary/Conclusions: We can conclude that, the inclusion of LoRRCa osmolar 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 INDUCTED 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.

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 targeted resequencing for six patients. Pyruvate Kinase (PK) enzyme activity assay were within normal limits in all these cases. All the mutations were predicted deleterious by PolyPhen/ SIFT/ Provean/ mutpmid and Mutatontaster. Mutations were validated in the parents/siblings (where available) to prove the mutational inheritance.

Summary/Conclusions: Unexpected PK deficiency were found after next generation sequencing analysis in the patients where PK enzyme levels were within normal limits. PK deficiency may be missed by conventional testing approaches. Our data demonstrates the clinical utility of next generation sequencing for molecular diagnosis. Timely detection of the cause in our patient is important to treat the patient in time and avoid the unnecessary transfusions in other patients. Identification of pyruvate kinase defects, if required, but therapeutically as well. A splenectomy (performed at appropriate age) can ameliorate the anemia in such patients and can eliminate need for transfusions in those that need them.
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 thermostablation TGT (Perkin Elmer) without any pretreatment and the resulting curves were compared with those of healthy individuals. Two groups of hereditary hemolytic anemias were considered: the hemoglobinopathies (sickle 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 CONSENSUS DELPHI INITIATIVE**

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**Background:** In the Western hemisphere, Gaucher disease (GD) type 1 is the most common GD phenotype, but the prevalence of GD type 3 is increasing. Symptoms and signs of the different GD phenotypes range 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 are 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 and administered. This categorization was checked and consolidated into summary factors by the non-voting co-chairs. In round 2, the factors were rated for importance by the panel using a 5-point Likert scale (1 = not important, 3 = important, 5 = extremely important). Any factors assigned an importance score of ≥3 by >75% of respondents were then rated for agreement in round 3, using a 5-point pivoted 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 overall 100% response rate in each round. Factors identified as major or minor in GD types 1 or 3 are given in the Table 1. There was 100% agreement that splenomegaly (≥3-fold enlargement) and disturbed occlu- lomotor function (slow horizontal saccades with unimpaired vision) are major factors in GD, and these were assigned a score of ≥3 in the prototype PSS; other signs and co-variables were assigned a score of 2. The panel was divided about whether severe anaemia, hepatomegaly, hyperferritinaemia and severe thrombocytopenia are 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:** 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.

**Table 1.**

**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 bioclinical 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 26.4±4.62, 14.6±1.51, and 0.86±0.81 respectively. According to clinical history of patients prior to diagnosis, 29 patients (20.7%) were diagnosed after acute hemolytic episodes. Of these patients 23, 4, 2, had hemolytic episodes due to drug, infection, chemical respectively. Subsequently, 78 (54.5%) and 27 (18.9%) of the remaining patients were diagnosed G6PD deficiency 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 (2.9%). Nearly half of the patients had hemolytic anaemia 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.
PB1846
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 idiopathic or secondary to underlying disorders with immune disturbance. Determining the optimal therapy is a challenge because of insufficient data from prospective controlled trials.

Aims: To evaluate the clinical characteristics, treatment responses and outcomes of our AIHA patients.

Methods: The clinical data of 32 patients with AIHA diagnosed and treated in our center between 2008 and 2016 were retrospectively analyzed.

Results: Median age 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 connective 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 danasumab. 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 3 patients who were treated with standard dose of Rituximab; two achieved CR and one did not achieve any response. One of two rituximab-responsive 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.

PB1847
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 lysosomes 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 mostly organized by the Clínicas de Referencia Nacional y Grupos de Expertos en Enfermedades Lisosomales (National Reference Clinics and Expert Groups on Lysosomal Diseases [EGLDs]), who created the Guías de Práctica Clínica (Clinical Practice Guidelines) for GD.

Aims: To evaluate the results obtained for 39 patients diagnosed with type 1GD (25 women and 14 men) through the National Reference Clinics and EGLDs.

Methods: This clinical case of 39 patients was analyzed and punched mutation of the β-glucocerebrosidase gene was determined. The patients were treated with imiglucerase enzyme 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 hepato-, splenomegaly, 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.
Gene therapy, cellular immunotherapy and vaccination

PB1849
DEMONSTRATION OF FUNCTIONAL SIMILARITY OF PROPOSED BIOSIMILAR ABP 798 TO RITUXIMAB
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Background: Proposed biosimilars undergo comprehensive structural and functional characterization before they can be studied in confirmatory clinical trials. ABP 798 is being developed as a biosimilar to rituximab. The originator is approved for treatment of non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, severe rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis.
Aims: ABP 798 was compared with rituximab sourced from the European Union (EU). Quality attributes assessed included binding properties (CD20, C1q, FcRn), 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, WI-L2-S, cell line. A direct binding ELISA was used to assess the binding of the Fc domain of ABP 798 to C1q. Binding of the Fc moiety of ABP 798 and rituximab to FcγRIa, FcγRIIa, FcγRIIb, and FcγRIIIa (158V) were evaluated in AlphaLISA competitive binding assays. Binding to FcRn was evaluated by an AlphaScreen competitive binding assay. ADC activity was evaluated in a functional cell-based assay, with CD20-expressing WI-L2-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 WI-L2-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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<th>0%</th>
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<th>40%</th>
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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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1PRAME expression level was increased in 10 fold after 1 hour and in 14 fold after 4 hour of incubation. The expression level increased in 2,7 and 7 fold under control, respectively, and MYD88 expression level increased in 1,1 and 2,5 fold under control. In THP-1 line the expression level (2,92% relative to ABL) and acute myeloid leukemia cell line K562 with high PRAME expression level (645%), acute monocytic leukemia cell line THP-1 with intermediate PRAME expression level (2,92% relative to ABL) and acute myeloid leukemia cell line NOMO-1 with low PRAME expression level (0,46%). All cell lines were incubated in RPMI 1640 with addition of LPS in final concentration 10 ng/ml. After 1 and 4 hour of incubation total RNA was extracted and PRAME and MYD88 expression levels were measured.
Results: After 1 and 4 hours of experiment in K562 cell line PRAME expression level increased in 1,1 and 2,5 fold under control. In THP-1 line PRAME expression level increased in 20 and 25 fold, respectively, and MYD88 expression level increased in 5,5 and 6,5 fold. In cell line NOMO-1 PRAME expression level was increased in 10 fold after 1 hour and in 14 fold after 4 hours, and MYD88 expression level was increased in 2,4 and 3,2 fold after 1 and 4 hours of experiment, respectively. Strong correlation between MYD88 and PRAME expression levels was observed (Pearson correlation coefficient 0,98).
Summary/Conclusions: We conclude that LPS after binding with TLRs initiates activating signal to PRAME gene via MYD88.

PB1851
MYD88 IN PRAME GENE ACTIVATION
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Background: PRAME is the most frequently expressed non-X-chromosomal cancer-testis gene in solid and hematological cancer. It is important, because PRAME often has a bad prognostic significance. In early studies was found that PRAME frequently coexpressed in translocation-harboring (like t(8;21), t(15;17) and t(9;22)) haematological diseases. Authors supposed that chimeric genes are activators of PRAME expression. But in large cases with normal karyotype PRAME is also expressed. Another reason for PRAME expression is promoter demethylation. But demethylating agents cannot activate PRAME expression in hematological cells taken from healthy donor. So presence of chimeric genes and methylation status only are not enough to explain why PRAME can be expressed in high level. Wadelin et al. found that PRAME expression level was increased in cell during lipopolysaccharide-treatment conditions. Role of MYD88 in this process still be unknown.
Aims: To check if MYD88 participates in activating PRAME expression in leukemia cell lines.
Methods: Three cell lines were used for incubation with anti-PRAME antibody: chronic myeloid leukemia cell line K562 with high PRAME expression level (645%), acute monocytic leukemia cell line THP-1 with intermediate PRAME expression level (2,92% relative to ABL) and acute myeloid leukemia cell line NOMO-1 with low PRAME expression level (0,46%). All cell lines were incubated in RPMI 1640 with addition of LPS in final concentration 10 ng/ml. After 1 and 4 hour of incubation total RNA was extracted and PRAME and MYD88 expression levels were measured.
Results: After 1 and 4 hours of experiment in K562 cell line PRAME expression level increased in 1,1 and 2,5 fold under control. In THP-1 line PRAME expression level increased in 20 and 25 fold, respectively, and MYD88 expression level increased in 5,5 and 6,5 fold. In cell line NOMO-1 PRAME expression level was increased in 10 fold after 1 hour and in 14 fold after 4 hours, and MYD88 expression level was increased in 2,4 and 3,2 fold after 1 and 4 hours of experiment, respectively. Strong correlation between MYD88 and PRAME expression levels was observed (Pearson correlation coefficient 0,98).
Summary/Conclusions: We conclude that LPS after binding with TLRs initiates activating signal to PRAME gene via MYD88.
Hematopoiesis, stem cells and microenvironment

PB1852
PD-1 IS HIGHLY EXPRESSED ON MEMORY T-CELLS RESIDING IN BONE MARROW BUT NOT IN PE-RHOPHILIC 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 cytotoxic T lymphocytes (CTL), central memory (Tcm) and terminal effector (Teff) memory T cells. We aimed to evaluate PD-1 expression on distinct memory T cell subsets in BM and PB of healthy donors.

Methods: The first portion of BM and a sample of PB were obtained from healthy donors (n=10, m=6, f=4) with age 37.5 (22-53) years old. Numbers of white blood cells (WBC) in BM and PB samples were evaluated by Sysmex XE-2100 hematology analyzer. 1*10^6 of WBC (excluded nucleated red blood cells) from BM and PB were stained using “lyse-wash-stain” standard protocol. The CD8-APC-Cy7, CCR7-PE-Cy7, CD28-PE, CD45R0-FITC, PD-1-APC antibodies on CD45+CD3+ cells were used for cell population identification 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%,8.03% in BM versus 10.4%,1.23% in PB. Similar trend was observed in other subsets: Tcm, Tcm, Tcm, Tcm, Tcm. Median of Tdm- CD8+ cells were 3.8%,1.015%, 22.7%,5.39%, 42.7%,7.86%, 21.9%,4.047% 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 might be critical for improving treatment of patients with cancers and hematological malignancies, but is still not well understood. Aims: To evaluate PD-1 expression on distinct memory T cell subsets in BM and PB of healthy donors.

PB1854
CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND METABOLIC FACTORS IN CHILDHOOD CANCER SURVIVORS
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Background: Circulating Endothelial Progenitor Cells (CEPCs) play a significant role in the maintenance of vascular integrity, balancing the 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 repair.

Aims: The study of CEPCs in children who received chemotherapy for Acute Lymphoblastic Leukemia (ALL) and solid tumors (ST) and the investigation of their levels in correlation with patients Body Mass Index (BMI) and blood pressure (BP) regarding the time following treatment.

Methods: Circulating CEPCs were defined as CD45-CD34+CD133+/VEGFR2+ and were 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 (≈30%). Moreover we detected CD45+ non-cellular particles by flow cytometry analysis. IKi-potent K562 cells are also take place. We also confirmed the fact BMSC can bear clonal genetic aberrations 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 ALL (22).

Summary/Conclusions: Our data stands for the existence of horizontal gene transfer between leukemia 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.

PB1853
BONE MARROW STROMAL CELLS MAY HAVE GENETIC ABBERRATIONS AND ARE CAPABLE TO GAIN THEM IN A CULTURE
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Background: Stromal microenvironment possesses 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 and tumor cells using serum-free membrane plate inserts 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 (patients) and RQ-PCR method. BMSC were examined 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 ALL (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=92%). 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 BMSC population (≈3,0 μm pores versus 20,0 μm cells as measured during microscopy). Besides BCR-ABL gene expression in BMSC was detected by RQ-PCR (BCR-ABL/ABL*100≈19%).

Summary/Conclusions: Our data stands for the existence of horizontal gene transfer between leukemia 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.
Results: The mean values of CEPCs subpopulation CD34+CD45negdimVEGFR2+ estimated in ALL, ST and Controls were 0.003860 (SE=0.00072), 0.00613 (SE=0.00146) and 0.002953 (SE=0.00004) respectively. The mean percentage of CD34+CD45negdimCD133+VEGFR2+ in ALL, ST and Controls was 0.00331 (SE=0.00072), 0.00499 (SE=0.00113) and 0.002663 (SE=0.00037). The correlation of CEPCs showed statistical significant difference of CD34+CD45negdimVEGFR2+ between the ST % BCR and CEPCs- (Mean Diff 0.003174, 95 CI of diff 7.716e-3 to 0.002672). In ALL the levels of CD34+CD45negdimVEGFR2+ the 1st year after treatment completion were 0.00458 (SE=0.00026), during 1-3 years 0.0031 (SE=0.00066) and >3 years 0.003423 (SE=0.00081). The levels of CD34+CD45negdimCD133+VEGFR2+ during the 1st year after chemotherapy was 0.00992 (SE=0.00371), 1-3 years 0.0027 (SE=0.00063) and >3 years 0.00331 (SE=0.00081). In the ST group the mean value of CD34+CD45negdimVEGFR2+ the 1st year after treatment was 0.0114 (SE=0.0048), 1-3 years 0.0047 (SE=0.0013) and >3 years 0.0036 (SE=0.0008). Whereas the percentage of CD34+CD45negdim CD133+VEGFR2+ the 1st year after chemotherapy was 0.00992 (SE=0.00371), 1-3 years 0.0034 (SE=0.00097) and >3 years 0.00336 (SE=0.00085). Statistical significant results were calculated for the levels of CD34+CD45negdimVEGFR2+ in ST group between the groups <1 year and over years’ post treatment (Mean Diff 0.007747, 95 CI of diff 0.0000041 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 highest levels of CEPCs were estimated in ALL and ST just after treatment completion with a gradual decrease as time passes. The highest percentages of CEPCs were evaluated in ALL patients with normal weight and blood pressure in contrast with the solid tumor group. Further investigation is necessary to highlight the importance of these data.

PB1855
HEMATOLOGICAL PARAMETERS IN NATIVE HIGHLANDERS OF LADAKH AGED 4-19 YEARS
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Background: High altitude (HA) has always intrigued physiologists because of the remarkable ability of man to adapt to the hostile environment. Hematological changes associated with HA exposure is believed to be driven by hypobaric hypoxia of HA. Majority of the studies on HA physiology and hematological adaptation have focused on the hematological adaptation in lowlanders visiting HA or have compared the hematological profile of native highlanders from Andes and Tibet with those of the neighboring lowlanders. These studies have mostly been directed towards adult population with no or little reference to children and adolescent age groups. Moreover these studies have been done mostly on the highlanders of Andes and Tibet with no data on Indian highlanders.

Aims: We aimed at assessing hematological parameters in native highlanders in the age group of 4- 19 yrs and compare the same with Indian lowland population as well as native highlanders around the world. We set up a reference pattern of normal values for evaluation of patients with a suspected MDS.

Methods: A total of 390 native highlanders of Ladakh in the age group of 4-19 yrs with no history of travel to lowland were taken for the study. A written informed consent was taken from the parents of all the subjects before starting interviewing them for the laboratory investigations. After taking antiseptic precautions, blood samples were drawn from the antecubital vein and complete hemogram including red blood cell indices were measured. The study subjects were stratified into five age groups (less than 5y, 5-8y, 10y, 10-12y, 12-15y and children more than 15y). Appropriate statistical analysis was done to compare the hematological parameters between the stratified age groups as well as between boys and girls.

Results: A total of 197 girls and 193 boys were included in the study. The mean age of the subjects was 128.8±80 (means±SD) months. The mean hematocrit value increased with age (38.6±2.5% in <5 yrs age group to 43.8±2.0% in >15 yrs age group). Similarly the mean corpuscular volume (MCV) also increased with age (MCV as percentage of highlanders around the world was 83±3.6 fl in <5y to 85±6.6±2.7 fl in >15y age). In contrast to the rising values of hematocrit and MCV we found that the mean corpuscular haemoglobin concentration (MCHC) decreased with age from 36.9±2.85% at <5 yrs of age to 33.7±2.3% at >15 yrs of age. The variations among the age groups are significant for hematocrit, mean corpuscular volume (MCV) and MCHC (p<0.01). On comparison of hematological parameters between boys and girls we found that the mean hemoglobin concentration in girls (13.9±0.29 g/dL) was significantly lower than boys (15.4±0.28 g/dL). The same findings were replicated in the mean RBC count (4.79±0.08 in girls vs 5.07±0.08 in boys). The mean MCHC in boys (37.23±0.93%) was significantly higher than those in girls (35.69±0.94%). The mean platelet count in boys was significantly higher than in girls (p<0.0003) (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 cytope- nias 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: multiple regression to analyse the dependence of BCS from the variables analysed.

Results: 134 cases were included. Panel 1 was applied in 106 cases (all centers) and panel 2 was used in 28 cases (3 centers). Age range: 10 months to 89 years. In the same age range, values for panel 2 were lower than those for panel 1. In multiple regression % BCP total cells-0.389 “log age” (years)–0.313 (for panel 2)+correction factor for labs +1.873. The correction factor for labs was 0 to -0.4. Age explained alone 49.6% of the variance of BCPs/total cells, while “laboratory” explained 5.2% and panel used explained only 0.8%. Age explained only 24.9% of the variance of BCPs/CD34+ cells.

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>% Total 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>62.1% (22.6-62.6)</td>
</tr>
<tr>
<td>7-18 years</td>
<td>1.4% (0.25-3.2)</td>
<td>40% (0.02-1.8)</td>
</tr>
<tr>
<td>19-35 years</td>
<td>0.84 (0.97-2.76)</td>
<td>13.5% (3.1-64.5)</td>
</tr>
<tr>
<td>&gt;36 years</td>
<td>0.7% (0.06-2.48)</td>
<td>16% (2.4-66.1)</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 equipments. Analysis by phenotype and as percentage of total cells was more accurate and less susceptible to variation.
Background: Disrupted hematopoiesis is life-threatening complication of allo-
geneic hematopoietic cell transplantation (allo-HCT). The interactions of haematopoietic stem/ progenitor cells (HSPCs) and bone marrow (BM) microenvironment, niche(s), control the homeostasis of BM. TGF-b induced gene 3 (BIG3H), 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 peristin 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 Hospi-
tal from January 2010 to August 2015. BM biopsy specimens at the time of day 28, day 90, day 180, 365 after allo-HCT were decalcified and stained with primary antibody of BIG3H and peristin. Expression of peristin in BM slides were reviewed by pathologist as follows: normal (0), minimal staining around blood vessels; (+1), sparse staining and/or focally staining; (+3), diffuse and strong staining, (+2), between (0) and (+3).

Results: The median age at transplant was 38.5 years (range, 17-68 years) and male was 13 patients (65%). Twelve patients (60%) were in CR1 (complete remission), 8% (4) in CR2. Thirteen patients (65%) received myeloablative conditioning regimen. The median dose of CD34+ cell was 3.67±10^6/kg (range, 1.6-7.67±10^6/kg). All patients achieved the neutrophil engraftment with a medi-
anum of 13 days (range 9-24 days). The median time of platelet engraftment was 15.5 days (range, 13-77 days). Idiopathic thrombocytopenia developed as follows; 13 patients at day 28, 16 at day 90, 6 at day 180, and at 3 day 365. There was no significant difference between idiopathic thrombocytopenia and the expression of BIG3H or Peristin (p=0.128). However, BM with thrombocy-
topenia manifested the low peristin/BIGH3 ratio (p=0.007). Acute GVHD was observed in 12 patients (60%) and chronic GVHD developed in 13 patients (65%). The development of thrombocytopenia does not differ according to acute and chronic GVHD (p=0.847) (Figure 1).

Summary/Conclusions: The peristin/BIGH3 might represent the status of
BM niche during the homeostasis and regeneration of hematopoiesis. High peristin/BIGH3 ratio could predict the recovery of the idiopathic thrombocy-
topenia.

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 by a confocal microscope with 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-
Grünewald-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 (210.4±42.1 ps). Normal shaped erythrocytes in smears of sickle cell patients showed similar values (214.6±3.1 ps), whereas crenated erythrocytes as well as drapenocytes revealed significantly elevated values (314.2±66.7 ps and 312.5±67.0 ps respectively). Regarding erythro-
poiesis, the cytoplasm of erythroblasts showed significantly shorter lifetimes (623.5±271.2 ps) than that of myeloblasts (835.9±198.4 ps) and the same was the case when comparing the nuclei (erythroblasts: 895.4±262.8 versus myeloblasts: 1166.4±287.9 ps). The same differences could be found in megal-
loblastic anemias. There were significant differences between the FLIM val-
es 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.

PB1860

TWO HEMATOLOGICAL MALIGNANCIES, SIMULTANEOUS OR CONSECUTIVE OCCURRENCE. EXPERIENCE OF A CENTER

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nina, Greece

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

PB1858

ASSOCIATION WITH OMENN SYNDROME AND CYSTINURIA: CASE REPORT

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Background: Omenn syndrome is one type of combined immunodeficiency, characterized with hepatoponemaglymphoma, lymphadenopathy, recurrent infections and has an austomal recessive pattern of inheritance. T lymphocyte count can be normal or decreased, whereas B lymphocyte count 6540/mm3, absolute lymphocyte count 1560/mm3, absolute eosinophil count 2270/mm3, absolute lympho-
cyte count 171mg/dl, IgA level 5,81mg/dl, IgM level 24,5mg/dl, IgE level 1270 mg/dl were found. T lymphocyte count 1092/mm3, B lymphocyte count 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 cyste-
zeine Stone. Perucaneous nephrolithotomy operation was performed, then the patient was given scholl solution. Stone analysis revealed to be cysteine 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, cysteine stones took form as a result of this alteration.
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. |  
| --- | --- |

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 only to bulky disease.

Methods: We retrospectively analyzed HL patients diagnosed in 4 centers in Tarragona area (Catalonia, Spain), between 1995 and 2015, treated uniformly according to a local protocol. Patients were assigned into 4 groups: G1: favorable early stage: ABVDx6 cycles, G2: Bulky early stage without other risk factors: ABVDx6+IFRDT. G3: unfavorable early stage (B symptoms) and advanced stage without bulky disease: ABVDx8, G4: Bulky advanced stage: AVBDx8+IFRDT.

Results: A total of 183 patients were analyzed with a median follow up of 82 months [range 1-244]. Male/female ratio was 1.29. Median age was 36 years [range 16-82]. Complete response was achieved in 160 patients (87.4%). The estimated OS at 20 years for the whole group was 62.7%. Kaplan–Meier method and log rank test were used for survival analysis. Cox proportional 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.
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 largest diameter. The standard of initial care was 6-8 cycles of ABVD followed by radiotherapy. Prognostic relevance of age more than 45 years, gender, CS IV, presence of B symptoms, IPS score, ESR<50 mm/h, Hgb<10.5 g/dL, WBC<15,000/mm³ and lymphopenia (lymphocytes <600/mm³ or <8% of WBC count) were examined.

Results: The median age of analysed patients was 37 (range 17-80). The median follow up was 98 months. For the whole group 5-year event free survival (EFS) was 63.1% and 5-year overall survival (OS) was 80.6%. In univariate analysis, worse OS was found in patients older than 45 years (5-year OS 66.7% vs 87.8%), patients with CS IV (5-year OS 70.2% vs 87.0%), B symptoms (5-year OS 77.1% vs 90.1%), 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>2 (5-year EFS 53.8% vs. 73.2%, [log rank; p=0.006] and lymphopenia (5-year EFS 50.0% vs 66.7%, kog rank p=0.025), while age, gender, B symptoms, ESR<50 mm/h, 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 age more than 45 years, ESR>50 mm/h and lymphopenia as independent prognostic factors for OS, while only IPS was identified as an independent factor for EFS. Afterwards, we performed survival analysis with aggregate scores of identified negative prognostic factors for OS for each patient. Since there was no difference in OS in intergroup analysis, groups were formed according to the score: 0, 1, 2, 3 is considered to represent complete metabolic response; score of 4, 5 – partial, no response or progressive disease.

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 advanced stage Hodgkin lymphoma (HL). More recently, the emergence of more sustained dose / intensity regimens like BEACOPP has improved the progression-free survival of patients, in particular due to better initial control of the disease. The choice between these two regimens of treatment is always controversial in particular because of an immediate and delayed toxicity potentially increased after BEACOPP. With the use of PET-scanner, escalation and de-escalation protocols based on the PET response were studied. Recent studies have shown the impact of the PET response to adapt the treatment: de-escalation after BEACOPP in case of a good response, escalation with more aggressive regimens like BEACOPP in case of poor response.

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 85%. We found no difference in survival between patients with negative PET and those with positive PET with 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, according to the Deauville score, was used to assess response in FDG-avid histologies using 5-point scale, both for interim analysis and treatment end assessment. The Lugano classification has proved extremely useful in the standardization of treatment response. A score 1, 2, 3 is considered to represent complete metabolic response; score of 4, 5 – partial, no response or progressive disease.

Results: By applying PET/CT results two pts’ groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). Using Deauville criteria complete response was observed in 95 (70.3%) HD and 94 (70.9%) NHL 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. This group of pts is found in increased risk category. Differences were considered for radiotherapy, while pts with more than one nodal or extranodal lesions after completion of standard chemotherapy were considered for high dose chemotherapyautologous stem cell transplantation (ASCT).

Summary/Conclusions: 18F-FDG PET was useful in HD and NHL pts after standard chemotherapy not only for determination of those who need additional therapy, but for the choice of the further management: radiotherapy, chemotherapy, or ASCT. A negative PET/CT study after the completion of therapy is an excellent predictor of good prognosis.
Background: Efficacy of PD-1 (programmed death-1) inhibitors in relapsed/refractory Hodgkin lymphoma (HL) has been established, but their role in relapse after allogeneic stem cell transplant (alloSCT) remains controversial due to the perceived risk of exacerbating graft-versus-host disease (GVHD). The literature is largely limited to case reports in patients with no or quiescent GVHD.

Aims: To determine the outcome of PD-1 inhibitor therapy and subsequent management in patients with concomitant biopsy proven active GVHD and progressive HL after alloSCT.

Methods: We describe the treatment and management of two patients in our centre.

Results: Case 1 had both extensive bony, lung and nodal HL with active skin, pleuropedicular and liver GVHD 6 months after donor leucocyte infusion (DLI) and immunosuppression withdrawal and 24 months after sibling alloSCT. Fifty% of the standard pembrolizumab dose (100mg) produced a PET partial response after 5 weeks but with concomitant biopsy proven, severe exacerbation of liver GVHD. The latter was managed with prednisolone, everolimus, ursodeoxycholic acid (UDCA) and subsequently tacrolimus with gradual but substantial improvement in liver function over the next 5 months (Figure 1) in the absence of further PD-1 blockade, but with progression of lymphoma. Pembrolizumab 50mg was then given with lymphoma response but again a significant (but less severe) flare of liver GVHD occurred. Subsequent 25mg doses failed to prevent lymphoma progression.

Reintroduction of 50mg doses approximately each 6 weeks for 4 doses with prophylactic everolimus, low dose prednisolone and ruxolitinib, has resulted in ongoing substantial but incomplete PET responses with associated stable liver GVHD. Case 2 had progressing mediastinal and pulmonary HL despite DLI-induced extensive liver and skin chronic GVHD 38 months post sibling alloSCT. Initial therapy consisted of optimisation of liver GVHD with 8 weeks of UDCA and prednisolone with improvement in liver indices (Figure 1). Pembrolizumab 50mg was then given, together with sirolimus and ruxolitinib as GVHD ‘ prophylaxis’, resulting 5 weeks later in complete metabolic remission on PET. Concomitantly liver GVHD was aggravated (See Figure 1) together with pancytopenia and marrow hypoplasia attributed to an immune-mediated phenomenon. Despite addition of tacrolimus and increased steroids, he remains with severe liver dysfunction and pancytopenia 10 weeks after the single dose of PD1 inhibitor therapy.

Figure 1.

Summary/Conclusions: PD-1 inhibitors can exert powerful graft vs HL effects even in patients with progression in the context of active GVHD, but at the expense of substantial GVHD exacerbation. Further exploration of approaches such as individualised dose titration according to response and GVHD activity and prophylactic therapy with non-calcineurin based immunosuppression which may not mitigate the anti-lymphoma effect will help evaluate whether durable responses with tolerable toxicity is possible in this context.
PB1868

PROGNOSTIC VALUE OF THE RED CELL DISTRIBUTION WIDTH IN PATIENTS WITH CLASSIC HODGKIN LYMPHOMA

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Background: The current gold standard for risk stratification in Hodgkin lymphoma (HL) is the International Prognostic Score. There are certain molecular and immunohistochemical prognostic markers in patients with HL, but their cost and technical constraints make such an application in routine impractical and expensive. Therefore, prognostic models for classic HL (cHL) that are inexpensive, simple, and easy to perform and interpret are needed.

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 > 14.5% (cutoff value calculated by receiver-operating characteristic) had a significantly worse two-year EFS (62.4% vs 90.4%, P = 0.009) but did not differ significantly in terms of OS (P > 0.2). Univariate analysis revealed that a high RDW (> 14.5%) was correlated with poor EFS (P = 0.019). Multivariate Cox regression analysis showed that RDW > 14.5% was an independent prognostic factor for EFS (hazard ratio [HR] 3.801, 95% confidence interval [CI] 1.14–11.45, 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 ameasure, 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 was to evaluate the impact of SM on the clinical course of LGLL.

Methods: This is a retrospective study of LGLL patients evaluated at Moffitt Cancer Center between January 1995 and May 2016. The diagnostic clinico-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: Of 668 screened patients with LGLL 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
indotify 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 74-86 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, fludarabine or alkylated agents based chemotherapy regimens +/- monoclonal anti-CD20 antibody. Validity of G8 was compared with standard relevant clinical inclusion criteria for treatment, laboratory parameters 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.

Results: Of all 89 patients median overall survival (OS) was 77 months, and disease free survival (DFS) in 58 (77.3%) patients achieving remission was 25 months. Among laboratory parameters, hemoglobin, platelet, neutrophil and monocyte count, as well as C-reactive protein, beta-2 microglobulin didn’t influence remission rate, ORR, OS and DFS. Elevated lactate dehydrogenase was found significant for CR rate, and low albumin level (<40g/L) for predicting OS. Among clinical parameters age, sex, presence of “B” symptoms, splenomegaly (>13cm), bulky disease (>10cm), extranodal (EN) disease, as well Charlson comorbidity index (CCI; ≥3), ECOG performance status (PS; ≥2) and G8 score were associated with worse outcome for both CR (P=0.005;HR 1.343, 95%CI:0.214-2.552) and OS (P<0.010; HR 11.262, 95%CI:3.03-4.400)

Summary/Conclusions: According to our experience, the implementation of G8 is good prognostic tool. Its incorporation into standard hematological indices may help in improving the optimal treatment approach decision in elderly patients.

PB1872
A PROSPECTIVE PHASE 2 TRIAL EVALUATING MONOTHERAPY WITH OFATUMUMAB FOR RELAPSED/REFRACTORY SPLENIC B-CELL MARGINAL ZONE LYMPHOMA (MORE TRIAL): SAFETY ANALYSIS RESULTS

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1B-cell Neoplasia Unit, IRCCS San Raffaele Scientific Institute, 2Strategic Research Program on CLL, IRCCS Ospedale San Raffaele, 3Università Vita-Salute San Raffaele, Milano, 4Hematology and Bone Marrow Transplant Unit, Ospedale Papa Giovanni XXIII, Bergamo, 5Department of Hematology, Niguarda Cancer Center, Niguarda Hospital, 6Department of Oncology and Transfusion Medicine Service, 6Unit of Lymphoid Malignancies, Department of Onco-Hematology, IRCCS San Raffaele Scientific Institute, Milano, Italy

Background: Due to the lack of prospective clinical trials, treatment guidelines for splenic marginal zone lymphoma (SMZL) are mainly based on chemotherapeutic expertise. Treatment options for progressive disease include splenectomy, chemo-immunotherapy, or anti-viral therapy in HCV-positive cases. As SMZL cells strongly express CD20 molecule, rituximab has been used in patients unfit for chemotherapy or splenectomy with high response rates. Ofatumumab is a fully humanized, high-affinity anti-CD20 monoclonal antibody that induced a more potent complement-dependent cytotoxicity if compared to rituximab. We designed this multicenter, open-label, single-arm phase 2 trial addressing activity and safety of ofatumumab monotherapy in patients with relapsed/refractory (R/R) SMZL.

Aims: The primary objective is the activity of ofatumumab in terms of complete response (CR) rate. Secondary objectives aim at evaluating the safety and tolerability and exploratory endpoints investigate biological features potentially related with response to ofatumumab.

Methods: All patients provided written informed consent. Key eligibility criteria included R/R disease after <2 prior lines of chemotherapy or immunochemotherapy (including single-agent rituximab). Patients are treated with ofatumumab (1st dose: 300 mg, 2nd-8th doses: 1000 mg) up to 8 weekly doses. Response assessment is scheduled 3 months after the last dose. Sample size was defined assuming a P0 of 45% CR, and a P1 of 65% CR. Per protocol the follow-up is 24 months. A minimum of 43 patients should be recruited. A safety analysis was planned after the enrolment of the first 10 patients. With an expected rate of adverse events (AEs) of 13%, if less than 3 AEs leading to withdrawal from treatment are reported, the accrual will...
continue to the planned 15 patients (interim analysis). Here we present safety analysis results.

**Results:** Ten patients (6 males, 4 females; median age: 69.5 years, 9–66 years, 1–65 years) were analyzed for safety. Eight patients were previously treated with rituximab, 26 adverse events (AEs) occurred in 7 patients, with only 5 grade 3–4 AEs. Ten AEs were drug-related, 30% were of grade 3 (Table 1). Three SAEs occurred: hypersensitivity, n=2, both related, dizziness, n=1, unrelated to study drug. No AEs leading to treatment withdrawal was reported and no patients died on study. Hematological and biochemical abnormalities included: neutropenia (any grade 6 cases, grade 3–4: 1 case), thrombocytopenia (grade 1–2: 3 cases), lymphopenia (grade 1–2: 2 cases), leukopenia (grade 1–2: 5 cases), 1 case of G6PD deficiency (grade 3, at baseline grade 2), 9 cases of ALP increase (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>
<thead>
<tr>
<th>Drug-related AEs</th>
<th>N of events (any grade 3–4)</th>
<th>Non-drug related AEs</th>
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<tbody>
<tr>
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</tr>
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<td>Thrombocytopenia</td>
<td>1</td>
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<td>Hypersensitivity</td>
<td>1</td>
</tr>
<tr>
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<td>1</td>
<td>Nausea</td>
<td>1</td>
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<tr>
<td>Dizziness</td>
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<td>Dizziness</td>
<td>1</td>
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<tr>
<td>Fatigue</td>
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<td>Fatigue</td>
<td>1</td>
</tr>
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<tr>
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<td>Fever</td>
<td>1</td>
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<tr>
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<tr>
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<td>Arthralgia</td>
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</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.

**PB1873**

**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 a record of FL in their electronic medical record (EMR). 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 >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, transition to DLBCL, or evidence of supportive care >30 days after end of LOT.

**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. Among patients who progressed after 1LT, 3 cases received 2LT; 3 cases received 1 single agent, and 65.7% received combination chemotherapy. 2LT regimens were similar to 1LT, with rituximab (18.9%) remaining the top single agent, while bendamustine+rituximab (25.9%) and R-CHOP (6.0%) remained the top combinations. Median (IQR) duration of 2LT was 3.6 (1.4–6.1) months. Of patients who reached progression after 1LT, 3 patients received 2LT; 3 patients received a single agent, and 64.4% received combination chemotherapy. In 3LT, rituximab (11.1%) was the most common single agent: bendamustine+rituximab (20.0%) and R-CHOP were the most common combinations. Median (IQR) duration of 3LT was 2.8 (1.4–4.7) months. Following 3LT, 26.7% (n=12) had evidence of remission, 39.9% (n=18) progressed, and 4.4% (n=2) had no evidence of remission.

**Summary/Conclusions:** FL treatment in routine clinical care aligns with treatment guidelines in 1LT and 2LT, with most patients receiving rituximab-based combination chemotherapy. Similar regimens were used in the 3LT setting. As expected, the rates of remission decreased with subsequent LOTs.

**PB1874**

**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 have bone marrow infiltration. The significance of BMB in indolent lymphoma remains controversial.

**Aims:** To analyze retrospectively the diagnostic accuracy of PET-CT in comparison with BMB in the initial staging of new FL in a single centre in daily practice.

**Methods:** One hundred and thirty-six patients with de novo FL have been diagnosed in our institution from June 2005 to October 2016. Of them, 64 who underwent both BMB and PET-CT before treatment were evaluated. The BMB was evaluated by hematologist and the interpretation of PET-CT images was interpreted by a nuclear radiologist. Positive BMB was defined as the presence of CD20+/CD10+ B-cells in 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 compared with 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>
<thead>
<tr>
<th>PET-CT</th>
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<th>TOTAL</th>
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<tbody>
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</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-

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**PB1875**

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A. Galaznik1,*, J. Bell1, L. Hamilton2, A. Ogbonnaya2, A. Raju2, K. Hennenfent2, M. Eaddy2, Y. Shou1
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 homecare, a large US EMR database, beginning 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 (89.0%) for 1LT induction 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
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Background: Results from phase 3 “Stil” and “BRIGHT” trials demonstrated the effectiveness of the combination Bendamustine-Rituximab (BR) compared to R in patients with advanced follicular lymphoma (FL). Bendamustine-+R is currently one of the most advanced Follicular Lymphoma (FL) strategies identified as a standard strategy in this subset of patients. However, only a few studies investigated the efficacy and safety of R maintenance in the FL 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 homecare, a large US EMR database, beginning 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 (89.0%) for 1LT induction 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

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 af 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 second- ary 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
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PB1879
ROLE OF F-18 FDG-PET/CT IN DETECTING LYMPHOMATOUS BONE MARROW INVOLVEMENT IN THE INITIAL STAGING OF PATIENTS WITH FOLLICULAR LYMPHOMA
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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, mean 32y), 48 FL (23 male, 25 female, mean 55y). Four of the 44 patients with HL had bone marrow infiltration in bone marrow biopsy (BMB+) and PET/CT detected bone marrow involvement in all of them. Patients, PET/CT was positive in bone marrow infiltration in 7 of the 40 patients without bone marrow infiltration in bone marrow biopsy (BMB-), these patients had bone marrow lesions on locations other than iliac crest. Six of the 7 patients were in advanced stage regardless of bone marrow involvement and a patient had sternal involvement by contiguity. Seven of the 69 patients with DLBCL had BMB+, 6 patients with DLBCL and 1 patient DLBCL and FL. PET/CT had detected bone marrow involvement in all of them. Sixty-two patients of 69 DLCL did not have bone marrow infiltration by biopsy(BMB-), but nine of them had BMB+. Seven of the 9 patients were in stage IV because of extranodal involvement of other organs. One patient had primary bone involvement without extranodal involvement by contiguity. Fourteen patients of 48 patients with FL had BMB+. Of these 14 patients with bone marrow involvement by biopsy, 5 patients had BMB+ and PET/CT could not detect another extranodal involvement in three of these five patients. Of the 34 patients without bone marrow infiltration by biopsy BMO-, 8 patients had PET/TAC+, and 6/8 could be classified in stage IV regardless of bone marrow involvement (Table 1).

Summary/Conclusions: Our series confirms that PET/CT is useful to detect bone marrow involvement in the initial staging of Hodgkin lymphoma and DLBCL. We avoid bone marrow biopsy in these histological variants of lymphoma. In follicular lymphoma, PET/CT did not detect more than one third of patients with bone marrow infiltration by biopsy. These results support the histological assessment of bone marrow in the initial staging of follicular lymphoma.
PB1880

PREDICTIVE FACTORS FOR INFECTIONOUS ADVERSE EVENTS IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA TREATED WITH BENDAMUSTINE-RITUXIMAB (BR) VS MAINTENANCE. RESULTS OF A RETROSPECTIVE MONOCENTRIC STUDY

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Background: The combination of bendamustine (B) and rituximab (R) is an effective and well tolerated treatment for B-cell malignancies. However, previous reports have shown a higher incidence of lymphopenia and secondary infectious complications in patients treated with BR than in patients treated with other chemoimmunotherapy 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 courses 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 the second tumors - were recorded according to the CTCAE v4.0 grade score. We computed the patients with at least one infection with a natural history characterized by continuous relapses.

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 stage disease (I-II: 37 (60%) had bone marrow involvement. Thirty four (46%) patients had follicular lymphoma, 17 (26%) mantle cell lymphoma, 11 (17%) marginal lymphoma, 5 (7%) diffuse large B-cell lymphoma and 4% other indolent lymphomas. Thirty two patients (49%) received BR as first line treatment, 51% as second line and above. Bendamustine was administered either at the dosage of 90 mg/m² 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 trimetoprim-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 4/2, cytomegalovirus reactivation in 2/22 and other infections in 10 patients. At univariate analysis, the infectious AEs were associated only with lymphopenia during the second cycle (p=0.043) and with neutropenia during the second, third and fourth cycle (p=0.026, p=0.003, p=0.018, respectively). No correlation with age, line of treatment and G-CSF administration was documented. Other AEs were: grade 3/4 neutropenia in 4/2, grade 3/4 lymphopenia (3%), grade 3/4 lymphopenia (80%). We reported also a 5% incidence of second tumors after treatment (lung cancer in 2 patients and prostate cancer in 1).

Summary/Conclusions: In our analysis, BR±R treatment confirms a toxicity profile similar to that reported in previous experiences. According to our results, an early lymphopenia and neutropenia (after two cycles) are predictive factors for infections AEs and for premature discontinuation treatment. Twenty 2% of patients discontinued treatment mostly because of the early withdrawal due to infectious complications. These data raise the question on the role of antibacterial, antiviral and primary G-CSF prophylaxis in all patients treated with BR.

PB1881

CAUSES OF DEATH OF FOLLICULAR LYMPHOMAS. MONOCENTRIC AND RETROSPECTIVE STUDY WITH A LONG PERIOD OF OBSERVATION

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Background: Follicular lymphomas are usually defined as incurable diseases with a natural history characterized by continuous relapses.

Aims: This study was launched to evaluate after a long observation period the causes of death during follow-up.

Methods: All patients with histologically confirmed diagnosis of follicular lymphomas grade I-II or I-IIa were selected from our data base starting from January 2000 until December 2004 in such a way to have more than 10 years of observation for the patients. We considered all patients with this diagnosis regardless to treatment and considering also patients followed with watch and wait. Patients were followed with ambulatory evaluation and for those lost to follow-up consulting the regional cancer registry.

Results: One hundred and forty-six patients were diagnosed and treated at our institution. The median age at the diagnosis was 61 years (range 21-82), large I-II in 47 patients, III-IV in 86. Bone marrow biopsy was positive in 87 patients, FLIPI 0-1 in 40, FLIPI 2 in 48, FLIPI 3 in 40 and FLIPI 4 in 18 patients. According to treatment 98 patients were treated with antracycline containing regimens, 34 with fludarabine containing regimens and 14 were observed or treated with rituximab. Ninety five patients had FLIPI ≥2 and 74 or chemotherapy combined in 24; 48 patients did not use rituximab. The median observation period for alive patients was 13.4 years (range 11-15 years) and 8 years (range 0.09-15 years) for dead patients. Fifty-six patients dead during this long period of observation and the causes were: 35 due to lymphoma progression (26%), 16 second neoplasms (25%), 12 other disease (18%), 1 car accident and 1 unknown. The overall survival with a median period of observation of 127 months (range 2-196) was 71%. In univariate analysis the best overall survival was statistically associated with low FLIPI score, the use of Rituximab and the obtaintion of complete remission. In multivariate analysis both FLIPI and the obtaintion of complete remission maintained the significance. Exactly the same results were observed if we considered the cause specific mortality.

Summary/Conclusions: In conclusion this retrospective monocentric study confirms that after a long follow-up period about half patients died of lymphoma and the other half died for complications related to therapy or to lack of immunological control (second neoplasm or other diseases). Follicular lymphoma confirms to be a good prognosis lymphoproliferative disorders and in the long observation period of patients clinicians must have maintained a careful evaluation of concomitant pathologies.

PB1882

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 subtypes, CT and PET/CT scans were studied. Extramedullary 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: Nineteen of 255 patients 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), 20 patients (22.7%) had follicular lymphoma (FL), 15 patients (17%) had marginal zone lymphoma (MZL), 6 patients (6.8%) had mantel cell lymphoma (MCL) and 2 patients (8%) that 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 was a significant association between INHL subtypes and transformation to NHL with unspecified INHL 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 Diffuse Large B-cell Lymphoma (DLBL). There was significant association between INHL subtypes and transformation to DLBL with unspecified INHL and extra nodal sites involvement. (P-value = 0.004). There was also a 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 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. MCL is associated with significantly lower mean survival time than other INHL 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 analyzed. Chemotherapy was the first choice of therapy until 2008, afterwards radiotherapy became the first line treatment for OAL.

Results: A total of 20 patients with OAL were identified. 60% (12/20) of patients were females with a median age of 61.5 years (range 45-85 years). 80% (16/20) had unilateral disease at presentation. MALT lymphomas comprised 75% (15/20), Follicular NHL 15% and CLL/LL 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 first 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% (6/20) were managed under observation. In the chemotherapy group 55% (6/20) received first line radiotherapy, 24Gy in 12 fractions in 67% (4/6), and 25% (6/20) were managed under observation. In the chemotherapy group 55% (6/20) were treated with first 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% (6/20) were managed under observation.

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, histologic 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 patients, 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, hematological 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 treated at the 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. Kruskal 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% PCFCL, 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<0.08). 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 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.
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 marginal zone 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 lacrimal gland (1.8%) and nodal marginal zone lymphoma (3 patients, 5.4%). Five of them presented with multifocal disease (8.9%). Fifty percent (28) had a clinical stage III / IV and 32 patients (57.1%) had a low risk of diagnosis (FLIPI 0-1). We found an antigenic stimulus in 11 patients (Helicobacter pylori, Sjögren’s syndrome, Hashimoto’s thyroiditis). The molecular study of MALT1 was performed in 25 patients and 3 presented the translocation (12%). Six of the seven cases (35.3%) showed IgH rearrangements. Antinuclear antibodies were positive in 15 of 32 patients (46.9%). At the time of the first treatment, 82 (91.1%) of patients achieved a complete remission (CR) (76.1%) and 10 partial remission (PR) (21.7%) after the first line of treatment. Among these, 17 received immunotherapy (37%), 10 immunotherapy (21.7%), 8 surgery (17.4%), 7 antibiotics (15.2%) and 4 radiotherapy (8.7%). We observed 7 relapses after the first line of treatment. Among these, 17 received immunotherapy (37%), 10 immunotherapy (21.7%), 8 surgery (17.4%), 7 antibiotics (15.2%) and 4 radiotherapy (8.7%). We observed 7 relapses (16.7%) vs 3 proctors (7.1%), achieving a CR in 7 (70%) and PR in 3 (30%) after rescue treatment. There was just one case of high grade transformation (1.8%), who was the only patient deceased in the series (1.8%), with a median follow-up of 70 months.

**Summary/Conclusions:** Marginal zone lymphoma is an indolent 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 males. Diagnosis is based on the examination of peripheral blood smear, flow cytometry and the bone marrow aspiration biopsy. Recently, B-RAFV600E mutation was demonstrated in 10% of Tiacci HCL case series.

**Aims:** Our aim is to investigate the frequency of B-RAFV600Emutation and other rare mutations of B-RAF (B-RAF436C, B-RAF601E, B-RAF249S) and their relation to clinical data and treatment responses.

**Methods:** Charts of 13 patients diagnosed with HCL were analyzed retrospectively. Patients’ clinical parameters were evaluated. HCL variant type patients were excluded. Paraffin blocks of spleen or bone marrow tissues are obtained from the pathology archives. One thin section (10 micron) of bone marrow or three sections of spleen are cut and DNA extracted by spin column technique, using DNA extraction kit. (Qiaamp DNA FFPE Tissue Kit, Qiagen) After spectrophotometric measurement of DNA; common and uncommon mutations of B-RAF were investigated. (Qiagen PyroMark Q24 system, Therascreen BRAFTKTK24, V1 (1/2) kit) Mutation and clinical data analysis were conducted using the SPSS 15.0 software. The study was approved by the local ethics board of Dokuz Eylul University.

**Results:** Male/female ratio was 9/4. Median age at diagnosis was 48 (37.59). Median follow-up time was 59 (3-96) months. At the time of diagnosis, 46.2% (n=6) of patients were asymptomatic. All of the patients had splenomegaly. Nineteen patients (33.9%) had monocytopenia and two patients (3.6%) had one dominant lymhadenopathy. Approximately half of the patients (%46.2) diagnosed with splenectomy. Only one patient was pancytopenic at diagnosis. Four patients were anemic (Hemoglobin<10 gr/dl), six were thrombocytopenic (Platelets<150000/µl). Leucopenia was seen in 84.8% (n=11) of the patients. Most of the patients (82.1%) had high positive anti-RAF antibodies (CA125, CA125α, B-RAF436C, B-RAFV600E, B-RAF249S), one patient had high positive anti-RAF antibodies (CA125, CA125α, B-RAF436C, B-RAFV600E, B-RAF249S) and two patients were positive for both mutations. No relation could be determined between clinical findings and mutation state.

**Summary/Conclusions:** B-Raf mutations are variable and common mutations in HCL patients. B-RAFV600E mutation can not be used as an indicator for patient selection that are appropriate for target therapies.

**PB1887**

**BENDAMUSTINE-RITUXIMAB IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA PREVIOUSLY EXPOSED TO RITUXIMAB. EXPERIENCE IN SEVEN HOSPITALS OF THE SPANISH LEMTADO 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 immunomothchemotherapy, in patients who are not considered refractory to rituximab, there is no standard treatment. In Spain, bendamustine in association with rituximab (BR) has not been reported for the indications. Nevertheless 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 association in a group of patients with follicular lymphoma previously exposed to rituximab.

**Methods:** Retrospective analysis of patients with relapsed FL treated with BR in 7 spanish hospitals on both the Spanish Lymphoma Group (GELTAMO). The study was approved by the reference Ethnic Committee and by all of the participating centres. All patients accession to the treatment through the compassionate use program.

**Results:** 41 patients were valid for analysis. Characteristics: 70% males with a mean age of 62 years (30-87). ECOGs 2 in 95% of cases, 73.2% in stages III-IV and FLIPI ≥3 in 48%. Bulky mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow involvement in 60% and 60% of the patients. Median cycles 5.1 (1-12) with a median cycle of 5.1 (1-12). Patients who received rituximab and received rituximab and 3 (7.3%) could be considered refractory. All patients received BR (B-90 mg / m2 D1-2, R-375mg / m2 D1). Median cycles 5.1 (1-12). Support with G-CSF was used in 27.5% of cycles. Maintenance with rituximab after obtaining a complete (CR) or partial remission (PR) was administrated in 42% of patients. Response: The overall response rate was 95.1% (65.8% CR-IR / 29.3% PR). With a median follow-up of 25 months (6-92) the median response duration was 41.9 months (32-85.1) and the median progression-free survival (PFS) was 67 months (27.4-86.5) with no impact neither by the number of previous treatments (1 vs 22) (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 neutropenia and anemia respectively. 59% presented febrile neutropenia. 43% received cotrimoxazole prophylaxis and 3 opportunistic infections were recorded (1 P. jiroveci 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 lymphoma (MALT) lymphoma is a rare disease however, the incidence is increasing and closely associated with helicobacter pylori (HP) infection. One choice of treatment of gastric MALT lymphoma refractory to HP sterilization is radiotherapy.

**Aims:** Our aim was to analyze the response to treatment with definitive radiotherapy in our department.

**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 one-day-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.

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**Infectious diseases, supportive care**

**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 and 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 LEUKAEMIA: 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 paresis. These patients are thus at high risk of development of new infections and reactivation of chronic infections. Despite the high prevalence of tuberculosis in the general population in endemic countries, it is rarely suspected and diagnosed in patients with acute leukemia.

**Aims:** To study the clinical manifestations of tuberculosis in patients with acute leukemia, as well as the impact of infection in the management of leukemia

**Methods:** A hospital database search was done to identify cases of acute leukemia and tuberculosis between a study duration of January 2013 to January 2017. All the medical records of the identified cases were retrieved from the central 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
incidence of multi-resistant bacteria (MRB) infections have notably increase. These infections are especially serious in hematological patients because of the immunosupression derived from their illness and their treatments. This increase is related to a high mortality rate and high health costs due to the severity of the infections and the difficulty in setting adequate therapy due to the lack of new antibiotics against these pathogens.

**Aims:** Define the MRB infections incidence and ways of presentation. As a secondary goal we try to determine if the isolation of these MRB has affected our empiric antibiotic therapy decision.

**Methods:** We retrospectively collected all positive blood stream cultures from hematologic patients from January 2012 to December 2016. We studied the characteristics, clinical features and pathogen isolates of our patients when the blood cultures were obtained.

**Results:** 1085 positive blood stream cultures were collected in 382 patients. The main characteristics of the patients are shown on Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td>1085</td>
</tr>
<tr>
<td>Patients</td>
<td>382</td>
</tr>
</tbody>
</table>

The infection source was: central venous catheter (CVC) in 48% of patients (including tunneled, non-tunneled and PICC lines), respiratory 10%, abdominal 8%, urinary 5%, skin/soft tissue 7% and multiple location 5%. Regarding CVC isolation’s, 11% were interpreted as contamination and 6% as colonization. Gram positive (G+) bacteria were more frequently isolated than Gram negative (G-) (72% vs 24%). Most common G+ bacteria was coagulase negative Staphylococcus, regarding G- E. coli, Klebsiella sp and Pseudomonas aeruginosa. MRB were detected in 6.1% of blood cultures being the most frequent G- (85%).

The main resistance mechanism was extended-spectrum beta-lactamases (ESBL) and carbapenemases (CP) production. BMR infections increased significantly in last year, mainly associated to CP, 0.5% in 2012 up to 7.1% in 2016 (Figure 1). 29.5% of MRB infections developed in patients with 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 India. All bone marrow aspirates and biopsies from HIV positive patients were retrieved and evaluated for the presence of infectious etiology. Cytological stains like Acid Fast Bacillus, Periodic Acid Schiff, Gomori Methenamine Silver and Mucicarmine were performed wherever needed.

**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 Cryptococcus, two cases of Leishmania donovani, and one case of Plasmodium falciparum was 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.

**Table 2.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>0.5%</td>
</tr>
<tr>
<td>2013</td>
<td>2.6%</td>
</tr>
<tr>
<td>2014</td>
<td>7.1%</td>
</tr>
</tbody>
</table>

**Figure 1.**
Overall mortality rate was 46.9%. The mortality for hematologic cancer was 75.2%. Excluding scheduled perioperative admissions, the records of 81 admissions of patients under 21 years old between May 2004 and August 2016 at Chonnam National University Hospital, Hwasun-gun, South Korea, were reviewed. The aim of this study was to report the outcome of pediatric cancer patients that result in admissions to the intensive care unit (ICU).

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. 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 is 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: The utility of bone marrow biopsy (trephine) (BMT) as a diagnostic tool in patients with fever of unknown origin (FUO).

Methods: We reviewed retrospectively the bone marrow biopsy results of the patients who underwent BMT from January 2010, to December 2016. Demographic, laboratory, diagnostic and outcome data were collected and retrospectively analyzed. We identified 31 patients who fulfilled the accepted classic 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 (6.5%) the histology revealed the presence of granuloma and/or lymphohistiocytic aggregates; one secondary hemophagocytosis (3.2%) and one mastocyttes infiltrate (3.2%). Six patients had a previous diagnosis of HIV/AIDS (19%). Subanalysis in HIV/AIDS patients revealed positive BMT culture in 2 of the patients (6.4%). Cultures demonstrated Mycobacterium tuberculosis and Mycobacterium avium intracellulare. There was one case in which a pathogen was grown in culture but that had a negative of 'direct examination'. The associations most likely related factor to contribute to the diagnosis in HIV/AIDS was male predominance (58% odds ratio [OR] 2.95; 95% CI, 1.19-4.25), clinical lymphadenopathy (OR 4.97; 95% CI, 1.90-2.44) or anemia (OR, 6.0; 95% CI, 1.72-21.3; P = 0.01), and one organ system dysfunction (OR, 18.5; 95% CI, 4.4-77.0; P = 0.001). Hematologic cancer patients had higher mean PRISM III score (16.5±4 vs 12.2±8.6; P = 0.51) and higher risk of sepsis (39.3% vs 13.0%; P = 0.17) compared 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 patients.

Efficacy and safety of tigecycline in febrile neutropenic patients with hematologic malignancies and carbapenem resistance: A multicentre retrospective study from chinese people

Tigecycline has broad spectrum activity against multidrug-resistant (MDR) bacteria, but few investigations of tigecycline in febrile neutropenic (FN) patients with malignancy are available.

Aims: This study attempts to investigate the efficacy and safety of tigecycline in FN and carbapenem-resistant patients with hematologic malignancies.

Methods: The study of 109 patients with hematologic diseases and FN were retrospectively analyzed. They are unresponsive to carbapenems for 3-5 days before receiving tigecycline (loading dose 100 mg; then 50 mg every 12 hours). Clinical response to treatment was defined as clinical cure, improvement or failure. Meanwhile, the adverse events were documented.

Results: The 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 hypotheetical eradication were 25.9% and 24.1%, respectively. The clinical effective rate was 85.7% when tigecycline was administered for more than 9 days, while just 48.3% when administered for less than 9 days (p < 0.001). Patients with bloodstream infection got a worse efficacy than those without (41.2% vs 69.6%, p = 0.024). For patients whose absolute neutrophil counts were less than 0.1 x 10^9/L, the clinical effective rate increased obviously (59.8% vs 86.4%, p = 0.019). The side-effects were well tolerated. No lethal adverse events were observed.

Summary/Conclusions: Our results demonstrated tigecycline was effective and safe for patients unresponsive to carbapenems with FN, combination and prolonged duration of tigecycline is recommended, and these results need to be further studied.
Methods: A retrospective cytological study of bone marrow aspirates from 95 patients with HMA (n=27), HMA+HIV (n=8), HMA+HIV/HEV (n=11) and HMA+intestinal parasitosis (n=49) has been performed.

Results: Bone marrow cellularity was normal in all the groups studied except in HMA+HIV patients, in which the cellularity was very diminished (statistically significant difference, p<0.01). Most frequent alterations observed in all samples (HMA and HMA+other entities) that could define the HMA-bone marrow cytological pattern were: - Erythroid hyperplasia with dyserythropoiesis, which is reflected in a decreased myeloerythroid ratio. - Increased eosinophilis 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 HMA samples). Quantitative results for these variables are summarized in Table 1. Lymphocytosis was significantly increased in HMA+HIV/HCV bone marrow (p=0.04). Significant detection of atypical lymphocytes (>4%) varied widely between the groups, ranging from 14.8% of HMA bone marrows to 75.0% of HMA+HIV bone marrows (statistically significant difference, p<0.001). There was no lymphoid evidence in any case. No quantitative or qualitative alterations were detected in megakaryocytes, except for a slight decrease in HMA+HIV bone marrows (statistically non-significant difference) (Figure 1).

Table 1. Quantitative results (mean±standard deviation).

<table>
<thead>
<tr>
<th>Reference values</th>
<th>HMA</th>
<th>HMA+HIV</th>
<th>HMA+HIV/HEV</th>
<th>HMA+HIV</th>
<th>HMA+IV/JP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelocytic/marrow ratio</td>
<td>3.1±0.8</td>
<td>2.2±1.3</td>
<td>3.4±0.6</td>
<td>2.5±0.7</td>
<td></td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>2±3</td>
<td>3±6</td>
<td>4±8</td>
<td>5±10</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>13±5</td>
<td>16±7</td>
<td>20±8</td>
<td>25±10</td>
<td></td>
</tr>
<tr>
<td>Plasma cells (%)</td>
<td>4±6</td>
<td>8±2</td>
<td>10±3</td>
<td>13±4</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: As far as we know, this is the largest series of HMA bone marrow analyzed. Identification of common cytological findings in all the groups studied allows defining a characteristic cytological pattern for HMA. 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 HMA 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.

PB1897

ACUTE APPENDICITIS IN LEUKEMIA PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION DURING THE NEUTROPENIC PHASE

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Background: Infectious complications arising from the gastrointestinal tract is common in neutropenic patients with hematologic malignancies, especially during 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 HSCT patients who occurred acute appendicitis during -10d~+60d in the Hematological Department of Nanfang Hospital from Jan. 2005 to July 2016 were analyzed. Patients were enrolled in our study based on the Modified Alvarado Scoring combined with ultrasonography (the MASS analytic). Aims was to combine the Modified Alvarado Scoring and ultrasonography to diagnose appendicitis during the neutropenic phase of HSCT.

Table 1. Combined with the Modified Alvarado Scoring and ultrasonography to diagnose appendicitis during the neutropenic phase of HSCT.

<table>
<thead>
<tr>
<th>Symptom*</th>
<th>Signa</th>
<th>Total Excravation</th>
<th>Blood Routine</th>
</tr>
</thead>
<tbody>
<tr>
<td>No nausea, vomiting</td>
<td>No tenderness</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>No abdominal distension</td>
<td>No guarding</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>No fever</td>
<td>No rebound</td>
<td>3</td>
<td>0.24</td>
</tr>
<tr>
<td>No pain</td>
<td>No rebound</td>
<td>4</td>
<td>0.37</td>
</tr>
<tr>
<td>No fever</td>
<td>No rebound</td>
<td>5</td>
<td>0.82</td>
</tr>
<tr>
<td>No fever</td>
<td>No rebound</td>
<td>6</td>
<td>0.82</td>
</tr>
<tr>
<td>No fever</td>
<td>No rebound</td>
<td>7</td>
<td>0.82</td>
</tr>
<tr>
<td>No fever</td>
<td>No rebound</td>
<td>8</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Reference standard by the Modified Alvarado Scoring System, Total scores were 10, Score 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. # "-": negative; "+": positive.

Summary/Conclusions: Acute appendicitis occurring during the neutropenic phase in HSCT patients could be diagnosed by the MASS and ultrasonography, and such cases could be cured by conservative therapy. This study could provide a further choice for the diagnosis and treatment of acute appendicitis in leukemia patients of HSCT.

PB1898

EPIDEMIOLOGY OF BLOODSTREAM INFECTIONS IN NEUTROPENIC AND NON-NEUTROPENIC PATIENTS WITH MALIGNANCY

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Background: Blood stream infections (BSI) in patients with malignancies remain associated with significant morbidity and mortality. The choice of an empirical antibiotic regimen is usually based on the local epidemiology of the microorganisms and their antibiotic resistance profile. Antibacterial guidelines for the management of sepsis in cancer patients in East Sussex Healthcare Trust (ESHT) recommend piperacillin/tazobactam as monotherapy and gentamicin 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 Coagu- lase negative Staphylococcus (CoNS) (22%), Klebsiella species (14%), Escherichia coli (13%), Streplococcus species (10%), Pseudomonas species (10%), Enterococcus species (8%) and Staphylococcus aureus (4%). In non-neutropenic patients, CoNS (29%), Escherichia coli (11%), Pseudomonas species (8%), Streplococcus species (7%), and Klebsiella species (5%) were isolated. Twelve Glycopeptide resistant Enterococci were isolated. Four Methicillin resistant Staphylococcus aureus were isolated. In addition, 15 Extended Spectrum Beta-lactamase producing Gram negative bacilli were isolated. Among Gram negative organisms, more than 91% isolates were sensitive to piperacillin/tazobactam, ceftazidime and ciprofloxacin and higher sensitivity was recorded in gentamicin and meropenem. Table1 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.
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 hypomethylating 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 serum blood culture for fever. Six patients were LymphoidAL and 94 were Myeloid AL. 41 patients were not eligible for intensive chemotherapy (for age and comorbidities) and were treated with hypomethylating 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.Haemoliticus 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 hypomethylating 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) tissues 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: reduced 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.

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.

Table 1. The sensitivity of antibiotic regimens used.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity</th>
<th>Neutropenic patients</th>
<th>Non-neutropenic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posaconazole plus amphotericin B</td>
<td>95%</td>
<td>100%</td>
<td>95%</td>
</tr>
<tr>
<td>Micafungin plus amphotericin B</td>
<td>95%</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>Calcitriol plus amphotericin B</td>
<td>95%</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>Ceftazidime plus amphotericin B</td>
<td>95%</td>
<td>95%</td>
<td>100%</td>
</tr>
</tbody>
</table>

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.) and renal toxicity and skin rash. Aims: We aimed at evaluating the real-life feasibility of a program of prolonged iron chelation in a population of acquired chronic anemia patients. Thus, we performed a retrospective analysis to evaluate which is the percentage of patients who in our centre actually receive and tolerate deferasirox treatment, among the cohort of eligible patients.

Methods: Deferasirox treatment is considered at our centre in patients affected by MDS or other forms of chronic anemia (excluded chronic bleeding) who fulfill criteria for iron chelation (high transfusion burden, ≥20 RBC units and/or a serum ferritin ≥1000 ng/ml). Starting dose is usually 10 mg/kg, titrated up to 20-30 mg/kg as tolerated. The cohort of patients transfused at our centre during 2015 and 2016 was considered for analysis. Causes of unsuitability and of treatment discontinuation were extracted from our database.

Results: Our cohort consisted of 58 patients, mainly affected by MDS (45 pts); other diagnosis were myelofibrosis (6 pts), NHL (2) and multifactorial anemia, 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.

Summary/Conclusions: 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 ±0.93 g/dl (range 9-16 g/dl). Overall, the prevalence of anemia in the entire population was 34.5%. Anemia was present in 53 (16.1%) of the children within group 1, 97/189 (33.5%) 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.

PB1903

IRON DEFICIENCY ANEMIA IN INFANTS AND YOUNG CHILDREN

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Background: Iron deficiency anemia in infants and young children is easy to be underdiagnosed. Anemia and iron deficiency are usually corrected after aged 2-3 years old, but it causes complications. There is an association between IDA and impaired neurocognitive function and exercise intolerance, even after treatment of IDA. Therefore, preventing the progression of iron deficiency is especially important during infancy and early childhood. When increased vulnerability is associated with rapid growth and development, especially of the brain.

Aims: To detect iron deficiency anemia early and to reduce the adverse impact by IDA, we assessed the characteristics of infants and young children with IDA, those at risk for IDA and those exhibiting associated characteristics of severe anemia.

Methods: Among 1,782 children with IDA aged 6 months to 18 years-old, we retrospectively analyzed medical records and laboratory data of 1,361 subjects ages 6–23 months with IDA who had been diagnosed between 1996 and 2013. We excluded patients with CRP ≥5 mg/dL.

Results: IDA was predominant in boys (2.14:1) during infancy and young childhood. Peak incidence was at 9 to 12 months of age. Only 7% of subjects were brought to the hospital with symptoms and/or signs of IDA, while 23.6% in subjects with severe IDA. LBW infants with IDA shows low adherence with iron supplementation. In a multivariate analysis, risk factors of severe anemia in infants included prolonged breastfeeding without iron fortification (odds ratio (OR) 5.70) and low birth weight (OR 6.49).

Summary/Conclusions: Many clinicians did not consider IDA as a real problem, so many children with IDA were not followed up. LBW infants need more attention to increase adherence of iron supplementation. For early detection of IDA, nutritional assessment should be evaluated in every infants and iron batteries in high risk infants (LBW infants, prolonged breastfeeding, picky eater and/or symptoms of IDA) at health screening visit.
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 world-wide. 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 flurometry 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 anaemia due to inflammatory diseases (AID).

Methods: We have analyzed in our lab, from 1st to 15th February 2015, all the complete blood counts (pediatric and adult) with anaemia (as defined by WHO) which had sedimentation rate (SR) and serum ferritin evaluations.

We have defined three different groups: IDA: Anaemia and Ferritin <20µg/L; AID: Anaemia, Ferritin >20µg/L and SR<20mm/h; Group control (GC): Normal levels of Hb adjusted by age and sex, as defined by WHO. Ferritin 20-120µg/L and SR<20mm/h. ZPP measurement was performed by hematofluorometry (AVIV, Biomedica, Inc.). Data were analyzed by SPSS v20.0 using Wilcoxon W and Mann-Whitney to examine differences between groups and receiver-operating characteristic (ROC) analysis to determine the cut-off values of ZPP. We considered a cut-off value of 20µmol/L as statistically significant and 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 32.3y in F [1-78], 28y in males (M) [1-78]; mean Hb was 10.6g/dL [SD 1.4]; mean ferritin was 9.3 µg/L [SD 4.85] and ZPP was 214.1 µmol [SD 121.3]; mean SR was 20.0 mm/h [SD12.9]; AID group: 75% F; mean age 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 [1-78], 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 78.6 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 69% 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 conclusion, the only 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 under-developed countries.
blood films are iron deficient pictures with the characteristic finding of reduced Haemoglobin (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).

Methods: An algorithm was designed in IT3000 to encourage testing and treatment for iron deficiency using a series of automated educational comments, while minimising unnecessary laboratory work. The impact that this algorithm had at WSCL was investigated by retrospective analysis of all the patient results from 1st November 2015 to the 1st of May 2016.

Results: In the first six months of operation, WSCL performed 232,192 CBCs and 30,204 blood films with an average review rate of 13.01%. Had this algorithm not been employed, 2,434 extra blood films would have been reviewed, bringing the review rate up to 14.05%.

Summary/Conclusions: Incorporation of an algorithm specific for iron deficiency in IT3000 has significantly reduced the review rate without any negative impact on patient care.

PB1907
THE RELATIONSHIP ENDOTHELIAL MICROPARTICLES AND ASYMMETRIC DIMETIL ARGinine IN CHILDREN WITH IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA
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Background: Iron deficiency anemia and iron deficiency without anemia increase the risk of atherosclerosis by increasing oxidative stress and inflammation. Endothelial dysfunction is an important factor of the pathogenesis of atherosclerosis.

Aims: Endothelial micro particles (EMP's) are considered as markers of endothelial dysfunction. Asymmetric dimetil arginine (ADMA) is known as another marker of endothelial dysfunction. In this study: we aimed to evaluate circulating EMPs and ADMA in children with iron deficiency and iron deficiency anemia and to disclose iron deficiency with the strongest relation with EMPs, ADMA and carotid atherosclerosis.

Methods: This study included 30 children with iron deficiency anemia, 30 children with iron deficiency without anemia and 30 healthy children whose anthropometrics measurements were recorded. Hemoglobin, serum iron level, iron binding capacity, ferritin, and lipid profile were studied. Circulating EMPs (CD144, CD146, and CD105) were measured by flow cytometry. ADMA was measured by ELISA. The carotid artery intima media thickness (CIMT) and left ventricular mass index (LVM) were measured using echocardiography.

Results: CD144 and CD105 EMP levels were lower in the iron deficiency without anemia group than in the control group and statically lower than in the iron deficiency anemia group (p<0.05). There were no significant differences in ADMA level between groups. Any significant variety in ADMA level was not observed between groups. CIMT was negative correlated with ferritin and high density lipoprotein and positive correlated with body weight.

Summary/Conclusions: In this study, endothelial dysfunction which occurs as a result of iron deficiency were observed. According to our result, CD144 and CD105 EMP levels in the iron deficiency without anemia group were lower than the iron deficiency anemia and control group; these levels in iron deficiency anemia group were higher than control group. In addition, when the level of ferritin has decreased, CIMT has increased. This study show that CD144 and CD105 may be related to endothelial dysfunction which occurs by iron deficiency.

PB1908
INVESTIGATION OF IRON METABOLISM FOR REGULATING MEGAKARYPOIESIS AND PLATELET COUNT ACCORDING TO THE MECHANISMS OF ANEMIA
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Background: Iron deficiency anemia (IDA) is characterized by depletion of total body iron stores. By contrast, chronic inflammation makes iron unavailable for hematopoiesis through a cytokine-mediated cascade, resulting in anemia of chronic disease (AOC). However, the laboratory data regarding the regulatory role of iron metabolism on platelet count has not been fully discussed yet.

Aims: In this study, we investigated the relationship between iron status and platelet production according to different anemic mechanisms representing different iron metabolisms.

Methods: The study included total of 759 blood specimens from 537 different patients. The complete blood count with various CBC index were measured using Advia 2120 (Siemens, USA). Biochemical indexes including iron level were estimated using Toshiba chemical analyzer (Toshiba, Japan).

Results: We found a significant relationship between platelet count and serum iron level in AOC group (p=0.27), whereas there was no correlation in IDA group. In AOC group, platelet count was significantly correlated to serum iron level only in AOC group with decreased serum iron level (p<0.0001), unlike AOC group with normal serum iron level.

Summary/Conclusions: Reactive thrombocytosis in inflammatory states may be a marker for increased iron metabolism. 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.

PB1909
SOLUBLE TRANSFERRIN RECEPTOR LEVELS OF APPARENTLY HEALTHY ADULTS IN PORT HARCOURT NIGERIA
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Background: Soluble transferrin receptor (sTfR) is an early marker of tissue iron deficiency before onset of anaemia. sTfR is one of the noninvasive tools for early diagnosis of iron deficiency anaemia, which is one of the most prevalent causes of anaemia in the developing world. However, there is no established reference range of this diagnostic marker sTfR in our environment. There is an important need to devise a screening tool for early detection of iron deficiency before onset of anaemia.

Aims: To determine the sTfR levels of apparently healthy adults in Port Harcourt, Nigeria, determine the reference range of sTfR in the study population.

Methods: This is a descriptive cross sectional study conducted at the University of Port Harcourt Teaching Hospital. one hundred and fifty participants (107 males and 43 females) who satisfied the inclusion criteria were enrolled in this study. The ages of the study population were grouped into four: 21-30, 31-40, 41-50, 51-60. Full blood count and sTfR concentration were assayed on anti-coagulated blood samples using a 3- part autoanalyser (sysmex -KX21N) and Human ELISA kit by biovendor respectively. The results were analysed using SPSS version 21. P value of <0.05 was considered significant.

Results: The mean sTfR concentration of the study population was 0.89±0.46ug/ml with a range of 0.3-3.05ug/ml. The mean value of sTfR for males and females was 0.92±0.49ug/ml and 0.82±0.37ug/ml. The mean sTfR for age group 21-30 was 0.88+/-.0.48ug/ml and 0.80+/-.0.47ug/ml for males and females respectively, while the mean sTfR for age group 51- 60 was 0.98+/-.0.53ug/ml and 0.97+/-.0.13ug/ml for males and females respectively. The mean sTfR levels did not differ significantly for age and sex.

Summary/Conclusions: This is the first cross sectional study in our environment to determine reference value of sTfR levels in healthy adults in Port Harcourt. This reference value was established as 0.3-3.05ug/ml. This study found no statistically significant relationship between different ages and sexes.
Myelodysplastic syndromes - Biology

PB1910

ROLE OF PRO-PHAGOCYTIC CALRETIULIN AND ANTI-PHAGOCYTIC CD47 IN MDS AND MPN MODELS TREATED WITH AZACYTIDINE OR RUXOLITINIB

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Background: Myelodysplastic syndrome (MDS) and Myeloproliferative neoplasms (MPN) are clonal myeloid disorders with the tendency to progress into acute myeloid leukaemia. Previous studies in solid tumours have shown an increase in expression of both pro-phagocytic calreticulin (CALR) and anti-phagocytic CD47, as they act in response to one another, reflecting a possible apoptosis vs survival mechanism in response to chemotherapy.

Aims: The aim of our study is to assess the changes in CALR and CD47 levels during treatment of MDS and MPN with 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-drugging occurring at 24 hours. Cells were then harvested, cDNA was synthesized for use in qPCR and protein levels determined by Western blot analysis.

Results: When treated with AZA, MDS cell models showed a 7-10 fold increase in CALR expression and 4-6 fold increase in CD47 expression. In contrast, the MDS/MPN intermediate 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 4-fold increase in CD47 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 studies have shown excessive rise in CD47 expression and low expres-

PB1911

GENETIC VARIANTS OF MSH3 AND BLM GENES MAY INFLUENCE MYELODYSPLASTIC SYNDROME SUSCEPTIBILITY AND PROGNOSIS

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous group of hematopoietic stem cell disorders, characterized by peripheral cytopenias, ineffective hematopoiesis and frequent transformation into acute myeloid leukemia (AML). Several genetic alterations are involved in disease development and progression as a consequence of stepwise accumulation of DNA mutations, which infers a defect in DNA repair mechanisms. Mutations in DNA repair genes of the nucleotide excision repair (NER) group, and affecting the mismatch repair (MMR), and DNA crosslink repair genes, among others, are the cause of inherited hematological disorders. The other hematopoietic stem cell cancers in these mechanisms have been identified for their potential role in cancer suscep-

PB1912

LOW RPS14 EXPRESSION IN MDS PATIENTS WITHOUT 5Q-ABERRATION SEEMS NOT TO BE RELATED WITH GENOMIC ALTERATIONS IN 5Q REGION

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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-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-

Aims: To evaluate the association of RPS14 expression in non-5q- MDS patients with 5q- MDS and its link with 5q- pathology.

Methods: We tested the association of RPS14 expression in non-5q- MDS patients with 5q- MDS and its link with 5q- pathology. In this work, we explore the origin of the altered RPS14 expression in non-5q- patients and its potential link with 5q-pathology.

Results: Non-5q-patients expressing low levels of RPS14 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 in a 7500 RT PCR System. β-glucuronidase gene was used as endogenous reference to normalize data. Samples were classified by RPS14 expression levels and differences in SPARC, CSNK1A1 and p21 expression mean values between the two groups were analysed using the Mann-Whitney U test. RPS14 and 32 genes related with MDS disorders were evaluated in relation with RPS14 expression. In order to establish if this group of patients could be beneficed by lenalidomide therapy, p21 expression levels were also analysed.

Summary/Conclusions: Although the important role of RPS14 in MDS pathol-
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 count (CBC), the fastest laboratory investigation, performed on the recently developed XN-10® analyzer. This study demonstrates that a simple CBC allows to diagnose MDS with 92% sensitivity and 81% specificity. When considering MDS over 50 years old, 4 patients had a significantly higher (% of ring sideroblasts and blasts; higher scores on IPSS, IPSS-R and WPSS; lower platelet counts, higher erythropoietin levels and greater expression of CD34 / CD117 / IL-12 / GicoP / IL-6 / TNFα) and molecular characteristics (methylation profile of genes p15, p16, DAPK, p15, R1, R2, R3 and R4 performed by PCR-MS, and evaluation of expression levels of regulatory molecules of apoptosis BCL-2, BAX, TRAIL, R1, R2, R3, FAS, Survivin, Caspase 3, Ct C, Glycoph and p53, by F).

Methods: In the 60-month follow-up, 11 patients progressed to Acute Myeloblastic Leukemia (AML), 7 with RAEB-2, 2 with RCMD, 1 patient with RAEB-1 and another with CMMML. These patients had a higher% of ring sideroblasts and blasts; higher scores on IPSS, IPSS-R and WPSS; lower platelet counts, higher erythropoietin levels and greater expression of CD34 / CD117 / IL-6. Assigning a value (+1) to each altered variable a new prognostic score was obtained, in which we used the novel Prognostic Score for Acute Leukemia. We observed that patients belonging to subtypes with the highest scores were those that progressed to AML, namely RAEB-1, RAEB-2 and RCMD.

Summary/Conclusions: In conclusion, we believe that this score may contribute to evaluate the risk of progression to AML, by reflecting the heterogeneity of MDS and its multifactorial pathogenesis. The coexistence of many altered variables not only contributes to the etiopathogenesis of MDS but also allows the assessment of potential leukemic transformation.

PB1915 CORRELATION OF PATIENT PROGNOSIS WITH PU.1 AND JDP2 PROVIDES POTENTIAL NOVEL PROGNOSTIC/DIAGNOSTIC MARKERS IN MDS

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Background: PU.1 is a key transcription factor in haematopoiesis that plays important roles in various haematological malignancies. Previously, significant down-regulation of PU.1 has been reported in high risk myelodysplastic syndrome (MDS) and acute myelogenous leukaemia (AML) patients. In order to clarify PU.1 molecular function we investigated the gene expression of PU.1 and JDP2 (c-Jun dimerization protein-2), a member of the activating protein-1 family located downstream of PU.1, in bone marrow from 12 MDS patients stratified according to IPSS-R score (6-low, 3-intermediate, 3-high risk), 1 AML patient and 10 normal controls. Methods: Samples were enriched for the mononuclear fraction by Ficoll separation. Total RNA was extracted and analysed by Real Time PCR for PU.1 and JDP2 expression relative to the housekeeping gene GAPDHusing the 2-ΔΔCT method. Western blot has been performed using anti-PU.1 and anti-JDP2 (Abcam)according to manufacturer instructions.

Results: We revealed both PU.1 and JDP2 were down regulated in MDS. In addition, our data suggests that PU.1 and JDP2 expression inversely correlates with disease, with expression of these genes consistently reducing according to IPSS-R groups. Furthermore, a positive correlation of PU.1 and JDP2expression <r<0.9333, p=0.0004 >, provides additional evidence that suppression of JDP2 by PU.1 could contribute to the pathogenesis of AML. Notably, PU.1 and JDP2 do not correlate to the same extent in normal HSCs, indicating that cofactors are required for PU.1 to exert its JDP2-regulating function and that such cofactors are not present under normal conditions. To confirm that JDP2 suppression is a direct result of reduced PU.1 we performed PU.1-knockdown in AML and stably expressing PU.1 short interfering RNAs versus control cells. These analyses reveal only a partial reduction in JDP2 expression when analysed by RT-PCR and Western blot, suggesting a more complex regulatory mechanism. Additionally, both PU.1 and JDP2 expression was recovered by treatment with azacitidine, which is routinely used to treat MDS, suggesting an involvement of JDP2 within the MDS cell cycle.

Summary/Conclusions: PU.1 and JDP2 expression correlates with patients prognosis highlighting a potential role as new diagnostic and prognostic markers in MDS.
PB1916
DECREASED EXPRESSION OF DECORIN, A WNT-PATHWAY RELATED PROTEIN, IN MESENCHYMAL STEM CELLS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES
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Background: Myelodysplastic syndromes (MDS) are clonal disorders of the haematopoietic stem cells (HSCs) characterized by inefficient bone marrow (BM) haemopoiesis and increased risk for leukemic evolution. Ineffective BM haemopoiesis in MDS has also been linked with an abnormal microenvironment that may sustain or even induce the aberrations within the HSC compartment. We have previously shown that the stroma progenitor cells, namely the mesenchymal stem cells (MSC), in MDS patients display impaired clonogenic and proliferative potential, reduced haemopoiesis supportive capacity and down-regulation of the canonical Wnt-signaling pathway.

Aims: Decorin, a small leucine-rich proteoglycan, and galectin-3, a member of b-galactosidase specific lectin family, are components of the extracellular matrix of the BM microenvironment. Both proteins have been implicated in the canonical Wnt-pathway participating therefore in cell growth and proliferation. The aim of the study is to assess the expression of decorin and galectin-3 in MSCs of MDS patients, evaluating their implication in the abnormal Wnt-signaling previously reported in MDS.

Methods: BM MSCs were isolated from 12 patients with lower risk MDS aged 51 to 75 years (median 67.5 years) and 12 haematologically healthy subjects aged 50 to 73 years (median 63.3 years), after informed consent. The study has been approved by the Ethics Committee of the University Hospital of Heraklion. BM MSCs were characterized according to international system for human cytogenetic nomenclature (ISCN) criteria, expanded and re-seeded for two passages (P). Total RNA was extracted from culture-expanded P2 MSCs and amplified by real-time PCR for the evaluation of decorin and galectin-3. Relative gene expression was calculated by the ΔCt method.

Results: A statistically significant decreased expression of decorin was identified in MSC of MDS patients (mean 1.338, SD 0.84) compared to the healthy individuals (mean 1.830, SD 0.71). (P<0.05). Galectin-3 expression was also decreased in MDS patients (mean 0.6758, SD 0.50) compared to controls (0.9395, SD 0.50), although not at a statistically significant levels.

Summary/Conclusions: MSCs from MDS patients display statistically significant decreased expression of decorin and a tendency towards decreased expression of galectin-3 in BM MSCs compared to healthy individuals. These preliminary data indicate that extracellular matrix proteins may have a role in the disturbed Wnt-pathway signaling and abnormal MSC function in MDS patients. The underlying mechanisms are currently under investigation.

PB1917
CLINICAL FEATURES, CYTOGENETIC STUDY AND OUTCOME OF ADULT MYELODYSPLASTIC SYNDROMES: REVIEW OF 101 CASES, A SINGLE CENTER EXPERIENCE IN ALGERIA
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Background: Myelodysplastic syndromes (MDS) are heterogeneous disorders defined as clonal diseases involving hematopoietic stem cells and even characterized by cytopenias, with a high risk of leukemic transformation. Morphological analysis of peripheral blood (PB) and marrow aspirates or bone marrow biopsies is the first step that ensures a diagnosis of MDS. Cytogenetic studies are important means of defining different prognostic groups and even of showing how patients are eligible for this or that treatment. We conducted the first study including conventional karyotyping and fluorescent in situ hybridization (FISH) for MDS in our country.

Aims: Our study was aimed to evaluate outcome of MDS regarding IPSS and IPSS-R classification in an emerging country.

Methods: Between January 2012 to December 2016, 101 patients with MDS were consecutively diagnosed. Frequent genetic abnormalities in MDS were screened by R-banding karyotype and metaphasic and interphasic FISH using a panel including six probes (5q-,7q-,20q-, del(17p13), MLL, Inv(3) t(3;3). 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 hematological blast cell was 80% (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 cell value was 4% (0-18). Cases were classed by cytology morphometry FAB as RA (n=45), REAB (n=34), RARS (n=16), other (n=6). Classification by WHO was 35% RAEB (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 anoma-
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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)/-7. The patients aged >65 and <65 were 70% and 30%, respectively. The patients aged >65 received induction chemotherapy with anthracycline-cytarabine combinations so as to continue with post-consolidation management with allogeneic transplantation, but the 44% died over the induction chemotherapy (OS: 2.2 months). The OS in patients aged <65 was 20.2 months in chemotherapy plus allogeneic transplantation. The OS in patients aged >65 was 10.3 months in the group of anthracycline-araC combinations, 3.81 months in supportive measures group (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/polo-like kinase pathway 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-T, and CMML), excepting patients at IPSS low- or interim-1 risk with respect to RA; ECOG PS of 0 to 2; no major organ dysfunction; and written informed consent. Treatment was administered intravenously for 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: 63-84) were enrolled, and 3 and 6 pts were eventually 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-T, RAEB-T, and RAEB-T, respectively. There were no DLT in each in the first cycle in low-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: 5 episodes of grade 3 non-hematological toxicities in 2 pts. One pt developed grade 4 infectious 1,200 mg arm, 2 cases each of grade 4/3 thrombocytopenia, grade 4 neutropenia, and grade 3/4 leucopenia, as well as 1 case each of grade 3 lymphopenia, development of organomegaly, pustular rash, and hyponatremia. 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 died from primary disease progression. Furthermore, 1 pt died of grade 5 bacterial pneumonitis that was rated to “Unrelated”. In the 1,200 mg arm, 2 cases each of grade 3/4 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 lymphopenia, grade 3/4 thrombocytopenia, and grade 3/4 neutropenia, as well as 1 case each of grade 3 lymphopenia, development of C-reactive protein, erythropenia, and hypochromia 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 324.8±83.9 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 being participated 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 front-line 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-2HIGH). Patients received subcutaneous azacitidine at 75mg/m(2) daily for 7 days every 4 weeks. All patients completed at least 6 cycles of therapy. 12/30 (40%) patients underwent more than 8 cycles of therapy. 18/30 patients underwent as well iron chelation therapy with deferasirox receiving a starting dosage of 10 mg/kg/day, subsequently titrated according to serum ferritin (SF) measured monthly.

**Results:** Complete response (CR), partial response (PR), and hematologic improvement (HI) were observed in 2 (7%), 5 (17%), and 12 (40%) patients, respectively. The median number of cycles to clinical response was 4 (range 4-8). The 2-year rate of transfusion of acute myeloid leukemia-free survival was 48%. Five serious adverse events occurred in five patients with one fatal outcome. 16 out of 18 patients who showed any hematologic response (CR+PR+HI) meeting International Working Group 2006 criteria had also performed deferasirox therapy. No increased toxicity was noted when deferasirox was used concomitantly with azacitidine.

**Summary/Conclusions:** Our results confirm the effectiveness of the therapy with azacitidine in HIGH-RISK MDS elderly patients with acceptable toxicity profile. Peripheral cytopenias were the most commonly occurring adverse event, with gastrointestinal adverse events and injection-site reactions among the most commonly occurring non-haematological adverse events. In conclusion, azacitidine is an important agent for use in the treatment of elderly patients with MDS. Furthermore concurrent use of deferasirox in patients with iron overload seems to significantly improve the hematologic response by reducing transfusion requirement.

**PB1921**

**EXPLORING THE RISK OF RED CELL ALLOIMMUNIZATION IN MEYLODYSPLASTIC SYNDROMES. TO WHAT EXTEND COULD CYTOTOGENIC 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 jaundice 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 226 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 [68 alloantibodies, 4 autoantibodies] with 42% of them developing more than 1 antibody. 5 additional patients developed autoantibodies without alloantibodies. Alloantibody specificities were as follows: E (22 cases), C (8), K (7), Cw/Jka/Kpa (5 cases each), Lu(a) (4), e/Fya (3 cases each), M(2), c/D Chido/Bga (1 case each). Collectively, alloantibodies against the Rh and Kell systems encountered the most with MDS-I(27.5%) and CMML (21.6%). In patients with MDS, 4 out of 8 patients with anti-C had also developed a secondary alloantibody specificities against other antigens, while 1 patient with CMML had developed a second antibody against Kell.

**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, disease subtype) did not have any statistical significance on the occurrence of alloimmunization and further studies are needed to investigate other possible risk factors. Prophylactic Rh and Kell antigen matched cells, when possible, would be a reasonable strategy until further knowledge is acquired.

**PB1922**

**PROGNOSTIC MARKERS THAT PREDICT THE OUTCOME OF REDUCED INTENSITY CONDITIONING TRANSPLANT IN ADULT PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A SINGLE CENTER EXPERIENCE**

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**Background:** Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic diseases, characterized by a clonal abnormality of hematopoetic 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. However, reduced intensity conditioning (RIC) regimen was used to reduce the toxicities associated with transplant procedure. The main concept of RIC rely upon adoptive immunotherapy especially in the low risk patients allowing the graft versus leukemia to occur.

**Aims:** This study aimed to investigate the outcome of allogeneic peripheral blood stem cell transplantation after the intensity conditioning (RIC) regimen for adult patients with MDS, the effect of different prognostic factors on outcome and the effect of chronic GVHD according to IPSS risk.

**Methods:** A retrospectively study analyzed the fifty-one patients with MDS who underwent transplantation at the BMT unit at Nasser Institute during a period of recent years. The data of RIC from HLA matched donors peripheral blood stem cell. Outcomes analyzed the incidence of acute and chronic GVHD, disease free survival (DFS) & overall survival (OS).

**Results:** They were 31 males (60.8%) and 20 females (39.2%). Their ages ranged from 17 to 60 years, with mean age±SD of 34.2±10.1 years. The incidence of patients with acute GVHD with RIC(myelo) in 14 patients (27.5%), MDS–U in 13 patients (25.5%), refractory anaemia (RA) in 12 patients (23.5%), refractory anaemia with excess blasts II (RAEB II) in 6 patients (11.8%) and MDS with hypocellular bone marrow in 4 patients (7.8%) and refractory anemia ring sideroblasts (RARS) in 2 patients (3.9%). According to IPSS, 11 patients (21.6%) were low risk, 28 patients (54.9%) were intermediate-I risk group, and 9 patients (17.5%) were intermediate-II & 3 patients (5%) were high risk group. The incidence of acute and chronic GVHD were 51.1% and 28.6% respectively. The 5-year estimate for DFS of the whole group was 21.8%. In univariate analysis, covariates associated with a better OS were recipient age <40 years (p=0.02) and the presence of cGVHD (p=0.002). On multivariate analysis the presence of cGVHD is significant predictor of survival (p=0.04). Also cGVHD significantly improve the OS for low and high risk MDS group (p = 0.02 and 0.03 respectively).

**Summary/Conclusions:** The presence of cGVHD significantly improved OS and reduced the risk of relapse in patients with MDS. We also found that the presence of cGVHD significantly improved OS especially in high-risk patients group, which suggests that the GVL effect may be beneficial in high-risk patients who do not receive intensive preparative regimens.
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transforming to ALL is not clear, further studies need to be performed. The reason why del(5q) seems to be present in a high proportion of MDS patients to confirm this hypothesis, and molecular examination is needed to characterise a cytogenetic event enabling the disease a switch from one phenotype (myeloid) to the other (lymphoblastic) could be a possible explanation for this phenomenon. 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 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 forms of MDS were diagnosed in 36.2% 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) and no improvement in 36.8% (variants RA, RCMD and RAEB). There was no response to treatment in 21.1% of patients (variants RCMD and RAEB). Positive effect on immunosuppressive therapy significantly more likely achieved in patients with hypoplastic forms MDS (57.9%) and the presence of clusters of lymphocytes in the bone marrow biopsies (36.8%). Dependence of treatment efficiency and cytogenetic 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.

PB1924

CHARACTERIZATION OF MYELODYSPLASTIC SYNDROMES WITH TRANSFORMATION TO ACUTE LYMPHOPROLIFERATIVE 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 lymphoproliferative 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, 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 described as 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, cytogenetic 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: KMT2A is known to be a gene involved in myeloid neoplasms as well as in acute lymphoblastic leukaemia. In a small series of cases we identified in the literature, 7 presented with del(5q) and 2 showed with anomalies of the 11q23 locus.

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 high risk of transformation into leukemia. MDS occurs in several subgroups that differ in frequency of appearance, the duration of the course and the probability of transformation into acute leukemia. The choice of therapy for a particular patient is determined by the morphological variant of the disease, the prognostic group, age and comorbidity. In hypoplastic cases of MDS are often used immunosuppressive therapy.

Aims: Analysis of the effectiveness of immunosuppressive therapy in patients with primary MDS Methods: The research included 19 patients with primary MDS from 22 to 58 years (median age 46 years, 11 male, 8 female). The diagnosis was made according to the criteria of the WHO classification of the MDS 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 forms of MDS were diagnosed in 36.2% 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) and no improvement in 36.8% (variants RA, RCMD and RAEB). There was no response to treatment in 21.1% of patients (variants RCMD and RAEB). Positive effect on immunosuppressive therapy significantly more likely achieved in patients with hypoplastic forms MDS (57.9%) and the presence of clusters of lymphocytes in the bone marrow biopsies (36.8%). Dependence of treatment efficiency and cytogenetic 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.
“intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) low” (n=20), 16.2 ng/ml in “intermediate” (n=15), and 21.7 ng/ml in “(very) high” (n=6), (p=0.701). Cytogenetic risk classification had no impact on median serum OCN levels (p=0.271). Summary/Conclusions: In summary, our cohort of patients with MDS, MDS/MPN and sAML show clearly decreased serum LD levels. The preliminary results suggest a tendency of serum LD levels to increase with higher risk categories supported by positive Kendall’s tau (p=0.210). Serum LD levels lie below normal limits, but seem not to be affected by disease risk. These results suggest specific hypotheses regarding the pathomechanism that shall be investigated on an enlarged data set, which we are continuously collecting.

PB1927

JUVENILE MYELOMONOCYTIC LEUKEMIA IN TURKEY: A RETROSPECTIVE ANALYSIS OF 65 PATIENTS

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Background: Juvenile myelomonocytic leukemia (JMML) is a chronic malignant myeloproliferative disease of early childhood

Aims: To define the status of juvenile myelomonocytic leukemia (JMML) patients in Turkey, in terms of diagnosis, clinical characteristics, mutational studies, clinical course and treatment strategies.

Methods: Data including clinical and laboratory characteristics and treatment strategies of JMML patients were collected retrospectively from pediatric hematology-oncology centers in Turkey.

Results: Sixty-five children with JMML diagnosed between 2002 and 2016 in 18 centers throughout Turkey were enrolled in 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, monocyte and platelet counts were, 32.9x10^9/L, 5.4x10^9/L and 58.3x10^9/L, respectively. Monosomy 7 was present in 18% of patients. JMML mutational analysis was performed in median serum OCN levels of 17.4 ng/ml in IPSS-R “(very) low” (n=17), 16.2 ng/ml in “intermediate” (n=15), and 21.7 ng/ml in “(very) high” (n=6), (p=0.701). Cytogenetic risk classification had no impact on median serum OCN levels (p=0.271). Summary/Conclusions: In summary, our cohort of patients with MDS, MDS/MPN and sAML show clearly decreased serum LD levels. The preliminary results suggest a tendency of serum LD levels to increase with higher risk categories supported by positive Kendall’s tau (p=0.210). Serum LD levels lie below normal limits, but seem not to be affected by disease risk. These results suggest specific hypotheses regarding the pathomechanism that shall be investigated on an enlarged data set, which we are continuously collecting.

PB1928

THE PRECURSOR B CELLS AS A PROGNOSIS FACTOR IN MYELODYSPLASTIC SYNDROMES

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Background: Recently, an immunosuppressive environment with low number of precursor B cells at the bone marrow has been related with poor survival in patients with very low/low and intermediate risk myelodysplastic syndrome (MS), but this negative impact is unclear yet.

Aims: The objective of this study is to establish if there is a negative association between the percentage of precursor B cells (%PBC) at the time of diagnosis of MS and progression-free survival.

Methods: We analyzed 48 patients with IPSS-R very low/low risk (VLL) and 34 patients with intermediate risk (INT) in the past 10 years in a single institution in Spain. We reviewed the %PBC at diagnosis measured by flow cytometry, and we calculated the time of progression-free survival (PFS) defined as the time between inclusion until progression to refractory anemia with excess blasts type 2 (RAEB-2) or acute myeloid leukemia (AML). The Competing risks regression test was used to assess the predictive value of PBC in relationship to PFS.

Results: Median age in both groups was 69 years, and median of progression to RAEB-2 or AML was 1.96 years in VLL group and 0.64 years in INT group. The %PBC was not a predictor of PFS in VLL group with a sub-hazard ratio (SHR) of 0.23 (95% CI: 0.003-13.96, P=0.485) neither in the INT group with a SHR 0.14 (95%CI: 0.001-4.52, P=0.211). We also performed a median split analysis to the%PBC with a median value of 0.1% in both groups. In the VLL group, patients with %PBC above the median had a median PFS of 2.48 years versus 1.99 years for the patients with%PBC below the median. In the INT group, patients with%PBC above the median had a median PFS of 1.14 years versus 0.83 years for the patients with%PBC below the median (Figure 1).

Figure 1. Summary/Conclusions: Our results not provide evidence in order to establish a prognostic value between %PBC at diagnosis in IPSS-R very low, low or intermediate MS.

PB1929

TO INFINITY AND BEYOND: NGS IN MDS

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Background: Myelodysplastic syndrome (MDS) constitutes a heterogeneous group of hematopoietic stem cell disorders, characterized by peripheral blood cytopenias in the presence of a dysplastic and hypocellular bone marrow. This 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. Genetic and epigenetic abnormalities are at the core of myeloid neoplasias development and despite the degree of dysplasia and blast percentages still being the main features for the WHO classification, a large amount of data has recently become available on recurring mutations in MDS, mainly due to massive parallel sequencing techniques.

Aims: Our aim was to search for genetic mutations in a cohort of patients with MDS.

Methods: We studied a total of 33 patients diagnosed with de novo MDS (WHO 2008 classification), using a Next Generation Sequencing panel comprising 45 myeloid genes.

Results: Patients were 15 male and 18 female, with a median age at diagnosis of 76 years (52 – 93 years). The MDS subtypes distribution was 16 patients (48.5%) with CMML, 4 patients with RARS, 4 with RAEB-1 and 4 with RAEB-2 (12.1% for each subtype), 3 patients (9.1%) with 5q-Syndrome and 2 patients (6.1%) with RCUD. These patients were stratified according to the IPSS as Low-risk (24.2%), Int-1 (33.3%) and Int-2 (18.2%), without any high-risk
patients. All patients required erythropoiesis stimulating agents and 9 patients required treatment with azacytidine (AZA), including all the Int-2 patients and 3 lower risk patients who progressed to a higher risk MDS. Estimated cumulative survival at 46 months was 67% with a median OS not reached and median follow-up time of 34 months. Patients receiving AZA revealed a trend towards survival benefit (mean survival 54.2 vs 50 months), independent of IPSS and R-IPSS. This finding is not statistically significant. Median follow-up time of 75.8% of patients had at least one gene mutation and it was most frequently related to DNA methylation genes (n=14), particularly in TET2 (n=7 patients) and DNMT3A (n=6 patients, 7 different mutations). We found a statistically significant difference between mutations in these genes and lower absolute neutrophil count (ANC) below 0.7 G/L (median 0.4 G/L, p=0.001). The most frequently mutated genes were related to signal transduction pathways (n=11; JAK1, JAK2, NRAS, CBL, GATA2, SH2B3, CSFR). Patients with these mutations had significantly lower serum EPO levels (p<0.001; median 32.35 vs 42.70 U/L). Furthermore, patients with such mutations demonstrated a clear discrepancy between analysis, with a median OS of 19 months for patients who reached in patients without mutations (p=0.001), being these results independent of the IPSS and R-IPSS risk groups. We were also able to identify a trend towards worst survival in patients with previously described high risk mutations (IPSS, EZH2, ASXL1, RUNX1 and ETV6 genes).

Summary: We conclude that the most frequently detected mutations were related to DNA methylation genes, as described in the literature, which was independent of the IPSS risk group, being observed in both low-risk and high-risk patients. These results raise the question whether hypomethylating agents may also be of benefit for lower-risk patients. We were able to identify a trend towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.

Summary/Conclusions: Our results are in agreement with those previously published regarding demographic features, distribution of pre-treatment cytogenetic abnormalities and prediction of survival. Myelodysplasias are among the most difficult hematologic 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 sample patients is of a major importance. This is especially true in our small population 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.

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 blast cell count < 5% of myeloblasts. This is usually an AML-like cytogenetic and anthracycline based, intensive chemotherapy (IC). 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/m2subcutaneously for 7 days), as bridge to transplant. Conditioning regimens used in MDS patients is busulfan based in younger patients (Bu-Flu, BU-Cy); in the elderly or less fit patients a RIC regimen (thiotepa 5 mg/kg e.v., fludarabine mg/m2x 3 and L-PAM 100 mg/m2) is administered.

Results: In the last ten years we performed 14 HCT (between June 2008 and September 2018) in patients with MDS with induction therapy. Median patients age was 63.5 years (range: 49-69), male/female ratio was 9/5. According to IPSS, 12 out of 14 patients were high-risk (2 int-2, 11, 1/4 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 were 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 state (CR vs noCR, p=0.22). In fact, 3 out of 6 patients treated with I.C. achieved CR in 1/4, 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.

Results: In the last ten years we performed 14 HCT (between June 2008 and September 2018) in patients with MDS with induction therapy. Median patients age was 63.5 years (range: 49-69), male/female ratio was 9/5. According to IPSS, 12 out of 14 patients were high-risk (2 int-2, 11, 1/4 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 were 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 state (CR vs noCR, p=0.22). In fact, 3 out of 6 patients treated with I.C. achieved CR in 1/4, 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.
Aims: Therefore, with the aim of assessing the safety and efficacy of ICT in the daily clinical practice, we retrospectively analyzed our single-center experience on ICT in MDS and other chronic anemias.

Methods: From October 1997, in our Institution, 69 pts (48 males), median age: 74 (23-96) yrs, with transfusion-dependent anemia, received ICT, because of a diagnosis of iron overload, i.e. both a transfusion history of at least 20 units of RBC and a serum ferritin (SF) higher than 1000 ng/ml.

Results: 40 pts (58%) were affected by lower-risk MDS (IPSS risk: low or intermediate-1), while 13 pts (18.8%) showed a higher-risk MDS (IPSS risk: high or intermediate-2) but were considered for ICT because of responsiveness to hypomethylating therapy and/or eligibility for allogeneic SCT. 16 pts (23.2%) were affected by other diseases (chronic myelomonocytic leukemia: 2 pts; idiopathic myelofibrosis: 3 pts; aplastic anemia: 9 pts; pure red cell aplasia (PRCA): 2 pts). 45 pts (65.2%) received deferasirox (DFX) as first-line treatment, 12 pts (17.4%) received DFX after a previous treatment with deferoxamine (DFO), while 9 pts (13%) received DFO and 3 pts (4.3%) received DFO after DFX because of contraindications to DFX or toxicity. Median time from diagnosis to the start of ICT: 18 months. Median number of RBC transfusions before the start of ICT: 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 ng/ml, 11 pts (15.9%) showed a drop of SF <500, 13 pts (18.8%) showed an increase of SF <500, in spite of ICT, and 18 pts (26.1%) showed an increase of SF ≥500. 12 pts (17.4%) achieved a SF value <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) (1 pt: 1.4%); gastrointestinal : 14 pts (20.3%) (grade ≥2: 1 pt: 1.4%); cutaneous: 2 pts (2.9%) (grade ≥2: no pts). Permanent discontinuation of ICT: 40 pts (58%), because of toxicity (16 pts: 23.2%), worsening of clinical condition (6 pts: 8.7%), discontinuation of transfusions (9 pts: 13%), allogeneic transplantation (9 pts: 13%). 5 pts (7.2%) (4 MDS and 1 PRCA) (with DFX: 4 pts; with DFO: 1 pt) showed an erythroid response following ICT, after 2, 4, 7, 32 and 112 months, respectively, and one of them (with PRCA) achieved complete remission. 35 pts (50.7%) died, because of infection (9 pts), AML (4 pts), cachexia (4 pts), other neoplastic diseases (3 pts), hemorrhage (2 pts), heart failure (2 pts), stroke (2 pts) and other causes (9 pts). 10 pts (14.5%) are still receiving ICT. With a median follow-up of 34 (2-230) months, median overall survival (OS) was 64 months for all pts, 51 months for MDS pts, 87 months for lower-risk MDS pts (50.7%) died, because of infection (9 pts), AML (4 pts), other neoplastic diseases (3 pts), hemorrhage (2 pts), heart failure (2 pts), stroke (2 pts) and other causes (9 pts). 10 pts (14.5%) are still receiving ICT. With a median follow-up of 34 (2-230) months, median overall survival (OS) was 64 months for all pts, 51 months for MDS pts, 87 months for lower-risk MDS pts (50.7%) died, because of infection (9 pts), AML (4 pts), other neoplastic diseases (3 pts), hemorrhage (2 pts), heart failure (2 pts), stroke (2 pts) and other causes (9 pts). 10 pts (14.5%) are still receiving ICT. With a median follow-up of 34 (2-230) months, median overall survival (OS) was 64 months for all pts, 51 months for MDS pts, 87 months for lower-risk MDS pts.

Summary/Conclusions: In conclusion, in our experience ICT appears feasible and effective, in terms of reduction of SF and OS, even in a population of elderly pts, if carefully selected.

Myeloma and other monoclonal gammopathies - Biology

PB1933

VCAM-1 AS A NOVEL DRUG THERAPY TARGET OF BONE MARROW MESENCHYMAL STEM CELLS IN MULTIPLE MYELOMA

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Background: Multiple myeloma is characterized by the clonal proliferation of malignant plasma cells in the bone marrow microenvironment. The pathogenesis of MM, in part, involves interactions between myeloma cells and the mesenchymal stem cells (MSC). The interactions between myeloma cells and bone marrow cells are establish through surface receptors (e.g. integrins, cell adhesion molecules, etc.), which determine tumor growth, survival, migration and drug resistance. Mesenchymal stromal cells modulate the pattern of myelo- ma markers on the cellular surface in vitro towards a less differentiated phenotype. However, the exact mechanism by which mesenchymal stromal cells carry out their functions is not yet fully understood.

Aims: To evaluate the effect of MSCs from healthy donors and myeloma patients over malignant plasma cells and the molecular changes produced for the suppression of MM.

Methods: Interactions between both cell types were studied through different co-cultures studies. We evaluate differences between culturing primary MSC and MM cell line RPMI 8226. Pathological MSCs were extracted from the bone marrow of newly diagnose MM patients. On the other hand, purified healthy MSCs will be isolated from donor patients. Pathological or healthy MSCs were cultured and co-cultured 24h after seeding with MM plasma cells RPMI 8226 for duplicates at 24, 48 and 72h. The phenotypical and molecular effect of the interaction of both cells were characterized by viability through trypan blue, cell apoptosis percentage (7AAD) and variations on expression of cell surface markers (MSCs: CD90, CD105, CD106 and CD54. MM cell: CD138, CD38, CD49d and CD11a) using flow cytometry, and statistically analyzed with GraphPad.

Results: We observed a decrease of apoptosis of MM plasma cells when are in co-culture with pathological MSCs at short-term (24h, 7AAD positive cells MM alone: 4.8%, MM in co-culture: 0.4%) and mid-term (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 10.7%) compared with MM plasma cells alone. However MM plasma cells not decreases the level of apoptosis at mid-term with healthy MSCs in co-cultures (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 18.0%). The molecular analysis showed a correlation between MSC lack of protection over MM plasma cells and the decrease in the levels of expression of VCAM-1 (CD106).

Summary/Conclusions: As reported in literature CD106 expression increase when MSCs are co-cultured with plasma cells. Adhesion of tumor cells to BMSC activates many pathways resulting in upregulation of cell cycle and anti-apop- totic proteins. MM pathophysiology is supported by a strong interaction between CD106/CD49d. Changes in VCAM-1 and VLA-4 expression have been demonstrated in cell lines assays, and were corroborated with primary cells in the context of MSCs protection over MM plasma cell. Thus, MM pathological MSCs did not change VCAM-1 levels and MM plasma cell protection be held. However, healthy MSCs have the capacity to modulate the VCAM-1 in mid-term to avoid the protection effect. Therefore, these results suggest MSCs VCAM-1 as potential drug therapy target in MM disease.
been implicated in putative downstream signaling of RAS, and may therefore provide a potential therapeutic target and drug resistance of MM cells.

**Aims:** We used shRNA-mediated knockdown of RaIA and RaIB isoforms to appraise their role as potential therapeutic targets and to analyze their connection to important signaling pathways, which regulate MM cell survival and proliferation. Because oncogenic RAS is a potential activator of the RaI pathway, we hypothesized that knockdown of RaIA and RaIB may contribute to the development and survival of RAS positive MM cells.

**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 RaIA or RaIB was achieved by electroporation of MM cell lines and the effect on cell viability was measured with flow cytometry using annexin V/propidium iodide staining. RaI pulldowns applied to test potential dependence of RaI activation on oncogenic RAS. Furthermore, RNA sequencing was performed to compare RAS and RaI gene expression signatures after respective knockdowns.

**Results:** Both RaI isoforms were expressed in primary MM cells and MM cell lines, with RaIA showing the most prominent and consistent protein expression levels. shRNA-mediated knockdown of RaIA strongly induced apoptosis in two thirds of the tested cell lines, whereas RaIB depletion did impair MM cell survival in less than half of the cell lines. Western analysis revealed no alteration of classical RalGDS/RalB signaling pathways after RaI knockdown. Ral activity appears to be independent of oncogenic KRAS or NRAS. In addition, RNA sequencing revealed differing gene expression signatures for RAS and RaI.

**Summary/Conclusions:** RaI 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 RaI may be important to identify useful clinical targets.

**PB1935**

**CXCR4 MUTATIONS FOUND BY USING DEEP SEQUENCING WITHOUT SORTING B CELLS, AND PROGNOSTIC IMPLICATION IN WALDENSTROM MACROGLOBULINEMIA**

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**Background:** Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytoid lymphoma with IgM monoclonal gammopathy. Most of WM harbor MYD88 L265P and one third of WM with MYD88 present CXCR4 mutations. Currently, frequency of CXCR4 mutations and its clinical implication is not reported in Asian patients with WM.

**Aims:** We investigated the profiles of CXCR4 and MYD88 mutation in correlation with prognostic implication. To detect minor cell population with CXCR4 mutation, we adopted a ultra-deep sequencing strategy for CXCR4, which can detect 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)]. Deep-sequencing for CXCR4 and interphase fluorescence in situ hybridization (FISH) for 6q deletion was performed on 31 patients with WM. Clinicopathologic features were compared among 3 groups according to MYD88 and CXCR4 mutation status (Group 1, MYD88WT and CXCR4WT; group 2, MYD88L265P and CXCR4WT; group 3, MYD88L265P and CXCR4Mutation; statistical comparison, Fisher exact test, one-way ANOVA).

**Results:** MYD88 L265P mutation was detected in 81.3% (26/32) patients with WM, 10.8% (9/83) in patients with DLBCL, 9.5% (6/63) in patients with CLL, 0% (0/15) in patients 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 cases with 6q deletion, 1 TEL/AML1, and 1 case with del(1p) were found. 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 3. Other clinical characteristics such as age, Hb, platelet, anaemia, hyperviscosity showed no significant difference among 3 groups. Group 1 showed adverse survival and 1 year survival rate of group 1 (68.7%) was lower than that of group 2 (93.4%), though it was not statistically significant (P=0.410). There were no death events in group 3 (MYD88L265P and CXCR4Mutation) 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 deep sequencing can efficiently screen a specific variant of CXCR4 mutation. 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.

**Figure 1.**

**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 1q23) under strong enhancer of IgH gene (1q42). 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 RISS. The p16 deletion, a characteristic of MM (Waldenstrom Macroglobulinemia, MMW) in approximately 10% of MM as having at least one of the following aberrations: deletion of 17p13 (TP53 gene), translocation t(14;16)(p13;q32) and translocation t(14;16)(q32;q23) determined by FISH. However, the unequivocal poor prognostic value of t(14;16)(q32;q23) was not confirmed in several MM series thus far, and further studies are needed.

**Aims:** The aim of our study was to assess the impact of t(14;16)(q32;q23) on event free (EFS) and overall survival (OS) in cohort of IgH/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 FIH). The basic FISH panel included 4 specific DNA probes (Abbott-Vysis, Kreatech and MetaSystems) detecting the IgH gene rearrangement (1), deletion 1q32 (RB1 gene/monosomy 13) (2), gain of 1q21 (deletion of 1p32) 3 and deletion of TP53 gene (4). Cases with rearranged IgH gene were gradually examined for 3 specific translocations- 1)(11;14)(q13;q32), 2) (4;14)(p13;q32) and 3) (t14;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 (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 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.
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 extent of the overall survival time of patients. However, the administration of some of the treatments may cause peripheral neuropathy or bortezomib, which is also associated with occurrence of a serious and common side-effect problem, which is the drug-induced peripheral neuropathy. The mechanism of the development of the peripheral neuropathy is poorly understood. Nevertheless, one of its potential cause, could be inadequate concentrations of crucial trophic factors, including neurotrophic and angiogenic factors, which are responsible for proliferation, differentiation, survival and death of neuronal and 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 4th induced in the course of MM treatment. 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.
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 disease stages (II and III). This deregulated expression of MEG3 promoted cell apoptosis and inhibited cell proliferation, migration and angiogenesis in MM ARP-1 and LP-1 cell lines. Furthermore, MEG3 increase the expression of phosphatase and tensin homolog (PTEN) and consequently inhibit MM cell proliferation and angiogenesis through sponging miR-181a in monoclonal (M) protein. A monoclonal serum M protein size at diagnosis was 12 g/L (1.7-28.5 g/L). IgG monoclonal clonality varied depending on the extent to which the FLC ratio was abnormal (the normal ratio). The median age at diagnosis of MGUS was 59 years (35-92 years). 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. 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 MICROFLUIDIC DEVICES

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Background: Cyogenic 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 cyogenic 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±6.5% in the marrow. After the MF-CD45-TACs enrichment, the detection rate increases to 10.3±0.4% in the group of RR MM, 1.67±0.31 in the group of ND MM, respectively, all significant increases compared to untreated samples.

Summary/Conclusions: We have developed a simple, rapid assay for improved diagnostics and risk-stratification for MM. With more precise diagnostics, the clinical outcomes of MM will be significantly improved.

PB1942

SERUM FREE LIGHT CHAIN RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: Monoclonal gammopathy 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 assessed the effect of expression of MDR genes such as MDR 1, MRP 1, BCRP, LRP on the effect of expression of MDR genes such as MDR 1, MRP 1, BCRP, LRP on the overall survival of patients with newly diagnosed and refractory/relapsed multiple myeloma and those with a normal ratio, and was independent of the size and type of the serum monoclonal (M) protein. Patients with an abnormal serum FLC ratio, non–IgG monoclonal clonality varied depending on the extent to which the FLC ratio was abnormal (the normal ratio).

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typing (IL-4, TGF-β1, IL-1α, IL-1β) was performed by PCR-SSP: study of cytokine genotypic abnormalities was performed by standard GTG-method and interphase FISH analyses with DNA probes: LSI 13(Ref13)14, IGHL/CCND1, IGHL/FGFR3, LSI TP53 (17q13.1); p-values less than 0.05 were considered statistically significant. Results: Previous results allow us to describe some cytokine genotype markers associated with the development of MM (IL-1α -889 TT, IL-1β -3962 TT, IL-6 -1747 GG and IL-6 n565 GG; gr. 1) as additional negative prognostic markers but IL-4 -33 CC and TGF-β1 codon 25 GG genotypes as additional positive prognostic markers (gr. 2). However, in some MM patients we found presence of negative and positive markers together (mixed markers; gr. 3). We analyzed cytoprofiles in MM patients with different prognostic markers in their genotypes (Table 1).

Table 1. Genotypes with prognostic markers

<table>
<thead>
<tr>
<th>Abnormal cytoprofle genotypes</th>
<th>Normal cytoprofle genotypes</th>
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<tbody>
<tr>
<td>Abnormal genotypes</td>
<td>Normal genotypes</td>
</tr>
<tr>
<td>1st gr. - MM patients with negative prognostic markers in genotype: IL-1α (C-308A) and IL-4 (C-589T)</td>
<td>IL-1α (C-308A) and IL-4 (C-589T)</td>
</tr>
<tr>
<td>2nd gr. - MM patients with mixed prognostic markers in genotype: IL-4 (C-589T) and IL-17A (G-174G)</td>
<td>IL-4 (C-589T) and IL-17A (G-174G)</td>
</tr>
<tr>
<td>3rd gr. - MM patients with mixed prognostics in genotype: IL-10 (C-197G) and IL-17A</td>
<td>IL-10 (C-197G) and IL-17A</td>
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</table>

The frequency of abnormal cytoprofle transformations in the 2nd gr. was noticeably lower compared to patients from the 1st and 3rd gr. (0.11 vs 0.87 respectively; p<0.05). Similarly, significant differences in the frequency between patients with positive prognostic markers and normal cytoprofle genetic profile (0.093) compared to MM patients with negative (0.22) or mixed (0.33) genotypes but normal cytoprofle profiles were also observed (p<0.05). In the 1st gr. the frequency of cytoprofle 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 presence of normal cytoprofle genetic profiles was remarkably higher (0.89) compared to patients with aberrations (0.11; p<0.05).

Summary/Conclusions: Thus, our results allow to describe IL-1α -889 TT, IL-1β -3962 TT, IL-6 -1747 GG and IL-6 n565 GG as markers associated with the presence of cytokine genotypic abnormalities in MM patient cells. However, IL-4 -33 CC and TGF-β1 codon 25 GG as normal cytoprofle genetic profile 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 cytoprofle 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. Such genetic factors include the presence in human genome of allelic variants of the regulatory regions of the innate immune response genes. At present time, they are considered as real risk factors for these diseases in a person with a certain set of genetic variants. Their distribution among the population corresponds to the population laws and has its ethnographic features. Analysis of the individual associations of genes polymorphism variants involved in the implementation of the immune response does not sufficiently complete answer about their role in the formation of predisposition to the development of chronic lymphoproliferative disorders (CLD) and multiple myeloma (MM). It is noted that in the pathogenesis of hematological disorders 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%) 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 TLR2 (rs5743708), TLR3 (rs3775291), TLR6 (rs5743810), TLR9 (rs5743836), IL1B (rs2856841), IL2 (rs2069762), IL4 (rs2243250), IL6 (rs1800795), IL10 (rs3087796), TNFA (rs34424920), TNFβ (rs2006295), FCGR2A (rs1801274) were performed by polymerase chain reaction with allele-specific primers (LifeTech, Russia). Analysis of interactions between genes was performed using nonparametric GMDR program (Generalized Multifactor-
Myeloma and other monoclonal 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.

Diagnosis of MM, response to treatment and degree of renal function recovery were based on the International Myeloma Working Group criteria. RI was defined as an estimated glomerular filtration rate (eGFR) <40 ml/min/1.73m², calculated by MDRD (Modification of Diet in Renal Disease) equation. Patients whose RI was explained by other causes were excluded. Early treatment was defined by initiation within 7 days after diagnosis. EM was defined as death within 3 months of diagnosis.

Results: MM was diagnosed in 52 patients, median age was 67 years (range 39-90), 61.5% were male, 38.5% had RI. The characteristics of the patients and front-line treatment are shown in Figure 1.

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 polyneuropathy 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 bz 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 the first year was infection in both groups.

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 and dialysis independence. Although toxicity 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 TRYPTASE POSITIVE MAST CELLS AND THE LEVELS OF IL-17, CORRELATE WITH ANGIOGENIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Angiogenesis in the bone marrow plays a very important role in the progression of multiple myeloma(MM). The procedure of angiogenesis is stimulated by several factors such as VEGF, FGF-2 and metalloproteases that are secreted from the tumor cells. The presence of IL-6 in the microenvironment, induces the production and the secretion of several angiogenic factors that activate inflammatory cells of the matrix, like macrophages and mast cells to secrete more angiogenic factors. IL-17 is among the most important cytokines that have an important role in the development of myeloma tumor. IL-17 is a proinflammatory cytokine that is secreted mainly by CD4 (activated memory cells) and stimulate macrophages, fibroblasts and other cells that release several cytokines. It has been reported that IL-17, induces angiogenesis in humans by stimulating the migration of vessel endothelial cells and adjusting the production of various proangiogenic factors. In a previous study, it was found that increased levels in stage II and stage III, resolved after therapy. Additionally, blocking the receptor of IL-17, with an antibody, cancels the effects of IL-17.

Aims: Aim of this study is to assess the relationship of the MCD and IL-17, in angiogenesis of MM, as well as their correlation with known angiogenic factors in disease progression.

Methods: We studied 52 newly diagnosed patients with MM, 32 women and 20 men, aged 67±9.6 years. According to the ISS stage, 19 were stage I, 17 stage II and 16 stage III. Regarding the type of paraprotein that had been found, 31 IgG, 17 IgA and 4 patients with light chains. 20 age and sex-matched healthy volunteers, were used as controls. Serum samples and bone marrow biopsy samples were obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study. We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. IL-17, bFGF and ANGIO-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 cells 400, using a graduated slide which corresponds to an area of 0.0625 mm². MCD was calculated as mean MCD / HPF.

Results: Statistically significant differences between patients and controls were observed in all measured parameters, MCD (p <0.001), bFGF (p <0.01) and ANGIO-2 (p <0.01), for IL-17 (p <0.04). 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 direct and indirect angiogenesis. IL-17 contributes to disease progression of MM also accompanied by increased angiogenesis in BM. In conclusion, mast cells and angiogenic factors seem to be important elements in the development of MM and become potential targets for the treatment and prognosis of the disease.

Figure 1. Characteristics of patients and overall survival according to renal function.
events drive HCRU, which may differ by treatment. Hospitalizations and hospital summary/conclusions:
Routine management of MM and treatment-related events.

Patients-per-month emergency room visits (18) and hospitalizations (78) higher had high mean per-1000 patients-per-month total visits (827), with per-1000 patients-per-month total visits were higher for PI+IMiD (876) than for PI (731).

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 (731).

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 (731) 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).

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).

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% 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 abnor-
mal (<0.125 or >8) serum free light chain (FLC) ratio (p=0.004), abnormal/nor-
mal 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. Sup-
pression 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 outpatients visit remain key drivers of HCRU in MM, which highlights an unmet medical need for effective therapy with better safety profiles.

The clinical characteristics and risk stratification of patients are sum-
marized 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 (731) 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).

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 was significantly associated with a highly abnor-
mal (<0.125 or >8) serum free light chain (FLC) ratio (p=0.004), abnormal/nor-
mal 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. Sup-
pression 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 lower level of normal) was significantly more frequent in SM 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 (18%) and it was significantly associated with severe suppression of the HLC-pair or of the other isotopes. After a median follow-up of 18 months (range, 6-35) progression to symptomatic MM was observed in 3 patients. All 3 had a diagnosis of SMM with an “evolving” pattern, highly abnormal HLC-ratio and severe HLC-pair suppression.

Summary/Conclusions: The findings presented in this study indicate that highly abnormal HLC ratio, severe suppression of the HLC-matched pair and other isotype HLC pairs are associated with known risk factors for disease progression in patients with high risk MGUS and SMM. The HLC assay could become a valuable tool in the risk stratification of these patients.

PB1953
EXTRAMEDULLARY MYELOMA IN THE “NOVEL AGENTS ERA”: OUTCOME, HETEROGENEITIES AND PECULIARITIES OF A COHORT OF 84 PATIENTS RETROSPECTIVELY ANALYSED IN A MONOCENTRIC EXPERIENCE
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Background: 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 (bEMD) and its relationship with soft-tissue related EMM (sEMD) in MM patients in our institution.

Results: 42 bEMD and 42 sEMD patients treated at Our Department between 2007 and 2016 were included in this study. Of the first group 10 presented EMM at diagnosis and 32 at relapse as well as 7 and 35 respectively of the second series. 31 among eEMD were dead and 11 were alive, 20 of bEMD patients were dead and 22 were still alive. EM was diagnosed using imaging techniques such as PET-CT (35%) or magnetic resonance MRI (65%). Biopsy was diagnostic if lesion was accessible (82%). The treatment was heterogeneous and all patients had received either thalidomide or bortezomib in the first-line of therapy. We showed that sEMD cohort has a significantly poorer survival compared to bEMD patients (median OS from diagnosis of EMD of 13 versus 58 months, P<0.001). Finally lung, liver (parenchyma-EM) and bone involvement (bEMD) was a worse survival outcome when compared to skin and lymph nodes masses respectively median OS of 12 and 10 months versus 18 and 15 months P<0.001. Conversely among bEMD group there wasn’t a significant advantage of outcome regarding the different bones involved. Kaplan-Meier estimates were used for survival analysis and differences between survival-times in patient subgroups were tested using the log-rank test (Figure 1A). Interestingly extramedullary-spread can be triggered by an invasive-procedures (surgery) or by a bone-fracture. In our population we have a case of breast-plasmocytoma diagnosed accidentally after a breast surgery. In this case the disease was documented by excisional biopsy confirming the disease.

Summary/Conclusions: The findings presented in this study indicate that extramedullary soft tissue disease has a poor prognosis especially in a relapse-setting. This work shows a significant difference in prognosis for different type of extramedullary-disease even between sEMD (better OS of skin and lymph nodes involvement) suggesting a different biological-behavior.

PB1954
DINAMIC PREDICTIVE FACTORS FOR A BETTER STRATIFICATION OF PATIENTS WITH R-ISS II NEWLY DIAGNOSED MULTIPLE MYELOMA
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Background: Revised International Staging System (R-ISS), combining the ISS score with cytogenetics and serum LDH, represents the most recent prognostic model for stratifying newly diagnosed multiple myeloma patients into three different survival groups. Although data for R-ISS development have been obtained from patients enrolled in clinical trials, this prognostic score has been validated also in real-life scenario (Tandon et al., 2017). In both non-clinical trial setting and IMWG experience, the majority of patients (about 65%) belonged to the intermediate risk group (R-ISS II) that, probably, needs better prognostication.

Aims: The aim of this study was to search for a closer stratification of MM patients with R-ISS II, taking into consideration dynamic aspects, such as therapeutic strategy and response to therapy.

Methods: We investigated the impact of variables, such as initial therapy, response to therapy and maintenance therapy, on PFS and OS in 108 newly diagnosed MM patients classified as R-ISS stage II, diagnosed between 2005 and 2015, who received novel agents such as immunomodulatory drugs and proteasome inhibitors. Score weights of the prognostic factors, found to be significant according to Cox regression model, were determined based on the regression coefficients.

Results: Median age of the 108 patients was 69 years (range 44-93) and 35% of them were older than 75 years. Thalidomide- and lenalidomide-based regimens were administered to 12% and 28% of patients, respectively, whereas 60% of the patients received bortezomib (54%) or carfilzomib-based (6%) regimens as induction therapy. Thirty-eight percent of the study population underwent ASCT and 40% received maintenance therapy. Regarding the response to the therapy, at least CR, VGPR and PR were documented in 35%, 66% and 87% of 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=1.6-45.0; 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 (24% vs 95% respectively) was observed in patients receiving maintenance therapy, compared to those who did not receive maintenance therapy (48% vs 20%; HR=1.9, 95%CI=1.2-3.3; p=0.010) whereas initial therapy did not affect the outcome. Assigning a value to the variables found to be significantly related to survival measures, according to the above methods, patients were stratified into the following two groups: low-risk (LR), including 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). Five-year PFS of HR patients was significantly shorter than the LR group (20% vs 58%; HR=2.5, 95%CI=1.6-3.8; p<0.001), whereas 5-year OS was 57% vs 80% (HR=1.9, 95%CI=1.1-3.3; p=0.021).

Summary/Conclusions: Our results suggest that in the R-ISS II MM patients,

Figure 1.
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 Interna-
tional Myeloma Working Group (IMWG). In addition to CRAB criteria, 3 bio-
markers of disease were introduced being (i) the presence of >60% clonal bone marrow plasma cells (BMPC), (ii) a serum free light chain ratio (FLC-ratio) >100 & (iii) the presence of >1 focal lesion on whole-body MRI (WBMRI). The intro-
duction 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, focusing on (i) the prevalence of these biomarkers, (ii) the diagnostic strength of BMPC esti-
mates using biopsy & (iii) the added role of dynamic contrast-enhanced WBMRI (DCEMRI) in the evaluation of SMM patients.

Methods: We retrospectively identified 28 SMM cases diagnosed between 01/01/09-31/12/14. Sufficient data for analysis was available for 25 patients. All patients underwent standard clinical & laboratory evaluation, bone marrow examination & WBMRI (T1- (+/-Gd) & T2-weighted sequences, diffusion-weighted sequences & additional DCEMRI sequences using time intensity curves).

Time to progression (TTP) is defined as time from diagnosis until MM development. Overall survival (OS) is defined as time from diagnosis until death from any cause. Survival curves 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 higher estimate of BMPC levels using biopsy (14.8%, SD 4.99) versus aspirate (6.45%, SD 6.59) (p=0.003). 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. WBMRI-positive was seen in 9 patients (36%). Progression was seen in 7/9 patients (78%) where only 1/16 WBMRI-negative patients (6.3%) developed MM (p=0.001). Median TTP was 19.9 months versus not reached for WBMRI-positive & (p=0.02). No additional BM biopsy (p=0.453). DCEMRI was positive in 14 patients (56%) thus identifying 5 additional WBMRI-negative patients with measurable bone marrow involvement. No significant difference concerning progression risk was however seen between WBMRI-negative patients being DCEMRI-positive (5/19, 26.3%) or -negative (14/19, 73.7%) (p=0.317). Median follow-up for baseline was 30 months revealed no significant difference concerning age, sex, genetic aberrations or the type of the monoclonal protein between both groups. In patients developing MM, progression was seen based on the development of anemia (5/6, 82.5%), bone pain (3/8, 37.5%), hypercalcaemia (1/8, 12.5%) & the development of punched-out lesions (4/8, 50%). No renal insufficiency was seen.

Summary/Conclusions: Our data shows that WBMRI-positive was the most frequent biomarker in a routine clinical setting. WBMRI-positivity, according to IMWG-criteria, clearly identifies patients with an increased risk of progression as was already shown previously. Although increasing the sensitivity of WBMRI, addition of DCEMRI-sequences didn’t have an added benefit. Our sample size was however relatively small. And although IMWG-guidelines do not state clear endpoints, our data suggests that a bone marrow biopsy cannot 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 risk of IMiDs-based regimens and the relevance of anticoagulant thromboprophylaxis.

Methods: Four hundred and one consecutive patients diagnosed with MM in a tertiary University Hospital between 1991 to 2015 were included. Data about VTE development, patient characteristics, myeloma-related factors, treatment and thromboprophylactic measures were retrospectively recorded. Multivariable correlates of VTE were assessed using Cox proportional hazards analysis.

Results: The median age at diagnosis was 66 years (range 24-90 years), and 47% were males. The results concerning treatment are extracted from 374 patients. The 164 patients that received IMiDs-based regimen, 27% did not receive any anti-thrombotic 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) (8%). Median follow was 40 months (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 on multivariate analysis, and the relevance of thromboprophylaxis has been proved, as the absence of this measure increased significantly the risk. Other factors that have also demonstrated to be independently associated with a higher risk for VTE were: BMI ≥30 kg/m2, prior Stroke or TIA, prior 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 addresses the survival analysis of IgM MM patients identified as having plasma cell disorder by the BD OneFlow system. The BD OneFlow solution for plasma cell disorders incorporates standardized assays, setup reagents, and protocols. The plasma cell disorders (PCD) panel is composed of the BD OneFlow PCST (Plasma Cell Screening Test) and PCD tubes and the EF liquid reagent system. Clinical results were identified as having plasma cell disorder based on clinical results.

Methods: Specimens for clinical results were collected from 81 patients. In 2 patients diagnosis of IgM MM was preceded by a 10-year period of monoclonal gammopathy of undetermined significance (MGUS) and in 4 patients (27%) diagnosis of WM was preceded by a 108, 78, 9 months period of IgM MGUS. Median overall survival in patients with a decreased HLC ratio in serum (by FreeLight) 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/FLC) immunoassays- FreeLight. Immunoassays and HLC/FLC ratios were concordant in all assessed IgM MM and WM patients. In IgM MM patients the detection of uninvolved polyclonal IgM revealed by 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 (IgM HLC<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 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.

PB1958

PB1959

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 discrimination of distinct cell populations by combining standard- ized 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 populations. In 1 patient 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, 78, 9 months period of IgM MGUS. Median overall survival in patients with a decreased HEM/NU but also between countries. The findings support the needs for the development of tailored clinical tools, educational activities and performance improvement interventions, adapted to the local context at a country level. Efforts should aim to address those current challenges in communicating with patients and educating them around their disease.

Aims: To compare the accuracy between the BD OneFlow PCD system and the EF liquid comparator.

Methods: De-identified remnant human bone marrow specimens (n=48) were collected at two study sites and tested in an unblinded manner within 24 hours of draw. Specimens were simultaneously stained with BD OneFlow PCD and BD OneFlow EF. BD OneFlow EF specific endpoint analysis was 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 EF demonstrates that BD OneFlow PCD is 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 system for aiding in the diagnosis of plasma cell disorders from bone marrow specimens. BD OneFlow PCD and BD OneFlow EFST are for in Vitro Diagnostic Use; CE Marked to the European In Vitro Diagnostic Medical Devices Directive 98/79/EC. 23-19565-00.
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. Inductive factors secreted by tumor cells and other cells of the narrow 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 processes evaded as the percentage of neoplastic plasma cells in the bone marrow 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 markers.

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 stage I, 11 stage II and 17 stage III. Regarding the type of paraprotein that had been found, 23 IGA, 5 IgG, 4 IgM, 2 IgD, 2 IgE and 1 IgA kappa and light chains. 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 evaluated as the percentage of neoplastic plasma cells stained with brown ting. Non-specific staining was observed at the other cellular components of BM.
(45%), and local discomfort for EMP. 52% of patients presented a MB at diagnosis, without significant differences between subgroups. In regards to treatment, combined therapy was the preferred option in the case of SPB (60%), whereas unimodal treatment strategies were more frequently used in EMP (86%). 11 of the 20 patients with SPB progressed to MM (55%) in a median time of 4 years, while none of the patients with EMP progressed (p=0.05). The 5-year PFS and OS was 61% and 90% respectively, 31% and 74% at 10 years. Although a tendency towards a higher PFE was observed in the EMP group, it was not statistically significant. No differences were found in PFS/OS between age groups (<60 or ≥60 years), axial vs appendicular skeleton location in SBP, type of treatment received, or the presence of MB. Furthermore, no association was found between the presence of MB at diagnosis and progression to MM (Figure 1).

Figure 1.

Summary/Conclusions: The age at diagnosis of SBP 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.

Method: Data from 1418 patients were analysed. KAPS analysis defined four risk 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, 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 1L (OS; 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 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, which is 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 worse 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 unfavorably 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 CD21/CD38/CD45/CD56/CD138 to distinguish and to enumerate MM cells. Monoclonal antibodies directed against CD20, CD28, and CD117 were used additionally. All samples were routinely tested for the presence of recurrent chromosomal aberrations, i.e. del 1p, amp 1q, del 6q, amp 15q, 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, elephant ear, or ISS stage. We found a strong association between lack of CD56 expression and light-chain only or asymptomatic myeloma. There was an association between CD28 expression and female gender (Table 1). In multivariate analysis including age, elevated creatinine, RISS, aHCT, 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 INAHCT and age. In patients not undergoing aHCT 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 for PFS only in the patient cohort not undergoing aHCT. As previously reported aHCT seems to abrogate the negative impact of CD56 negativity. We propose CD56 expression to be used as a prognostic marker in patients with RISS 2 stage disease and to be available for these patients should undergo aHCT.

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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. These data were collected on an emergency department visit and in regular checkups 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 relapse 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 69 years was not significantly different between health care providers in 2015 and 2016 (2015: 47±14%, 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 OBP (2015: 45% / 2016: 26%). In first-line treatment, 52% of patients eligible for SCT received mobilization chemotherapy in addition to induction therapy. More than 90% of transplanted 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: 16%, 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 collect three autologous transplants, enabling a possible tandem ASCT and ASCT for relapsed disease.

PB1967

MODIFIED HYPERCVAD PLUS BORTezomib-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-hyperCAD (bort-CVAD)’. We present data from a retrospective study in 33 patients with relapsed and/or refractory multiple myeloma treated at Oregon Health and Science University.

Aims: The primary objective is to describe the safety and efficacy of the hyper-CVAD regimen with vincristine or bortezomib in patients with relapsed or refractory MM treated at Oregon Health and Science University.

Methods: IRB approval was obtained to perform this retrospective analysis. We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RRMM). Patients who received 1 cycle of mod-CVAD (n=15) or bort-CVAD (n=18) from Jan 1 2011 and Dec 31 2015 at the Knight Cancer Institute were included. Most patients were previously treated with/refractory to proteasome inhibitors (97%/86%) respectively. 13 received prior autologous stem cell transplant (auto-HCT), the median number of prior lines was 3 (range 1-8). High risk cytogenetic factors t(4;14), t(14;16), or del 17p were present in 8 and extramedullary disease in 13 patients overall. Randomized patients contained cyclophosphamide 300 mg/m2 IV every 12 hours for 8 doses; doxorubicin 9 mg/m2/day continuous IV infusion every 24 hours and dexamethasone 40 mg by mouth on days 1-4; vincristine 0.4 mg/m2 IV infusion every 24 hours on days 1-4 (mod-CVAD) or bortezomib 1.3 mg/m2 SQ on day 1 and 4 (bort-CVAD). All patients received MESNA 350 mg/m2 IV every 24 hours on days 1 through 4; granulocyte colony-stimulating factor 24-48 hours following the completion of chemotherapy; and standard infectious prophylaxis. International Myeloma Working Group uniform response and European Society for Blood and Marrow for minor response (MR) criteria were used.

Results: The median number of cycles was given was 2 (range 1-6). Cycles were repeated every 3 to 4 weeks. Median follow up was 48 and 33 months in mod-CVAD and bort-CVAD respectively. The ORR was 40% in the mod-CVAD group: 6 partial (PR), 6 minor (MR), and 3 stable disease (SD) compared to 44.4% in the bort-CVAD group: 1 complete response, 7 PR, 2 MR, 6 SD and 2 progressive disease (Fishers exact p=0.80). A total of 13 patients proceeded to autologous stem cell transplantation (tandem ASCT). The median overall survival time was 6 and 11 months respectively, which was comparable between arms (Log rank test p=0.6635 and 0.7369). New or worsening of peripheral neuropathy occurred in 2 patients in the mod-CVAD and bort-CVAD groups respectively. There was no statistically significant association between treatment and febrile neutropenia (Fisher’s exact test P=0.15). There were no statistically significant differences in safety and tolerability between treatment arms. Three and 6 patients in the mod-CVAD and bort-CVAD arms discontinuation therapy due to toxicity or treatment complications respectively.

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 been used the combination of lenalidomide and dexamethasone (RD) for RR-MM patients after 2010. Therefore, we analyzed efficacy and safety of RD in Turkish patients with RR-MM.

Aims: We aimed to evaluate the outcome and the tolerability of the RD in patients with RR-MM who had been treated under the standard clinical practice between October 2010 and June 2016.

Methods: This is a retrospective, single center study. Patients’ clinical and laboratory data were collected from patient’s files. The overall and progression free survival (OS and PFS) were evaluated. OS and PFS were calculated according to the Kaplan-Meier method. Log-rank test was used to evaluate the variables affecting OS and PFS (univariate analysis).

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, patients receiving RD for more than 12 cycles. Adverse events occurred in 69 of patients (57.5%). Hematological and non-hematological adverse events were found at the same rate (n=47, 39.2%). The treatment discontinued was due to AEs in 11.7% (14 patients). The overall incidence rate (IR, events per 100 patient-years) of second primary malignancies (SPMs) was 0.93 (95% CI, 0.04- 4.60). The rate of anemia was 12.5% and thrombocytopenia was 9.2% in all grades. Penumania (15.8%), fatigue (14.2%) and herpes infections (0.8%) have been reported as most frequent non-hematological side effects.

Summary/Conclusions: RD is a safe, well tolerated and effective treatment in patients with RR-MM. Good response, previous ASCT and using more than 12 cycles are associated with better survival. Higher OS and PFS and ORR seem to be related to using RD in the first relapse. Adverse events are manageable and lower with prophylaxis.

PB1969

OPTIMIZING THE MANAGEMENT OF NON-HEMATOLOGICAL ADVERSE EFFECTS RELATED TO LENALIDOMIDE IN RELAPSED MULTIPLE MYELOMA PATIENTS. ONE CENTER EXPERIENCE

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Background: During many years, the combination of lenalidomide and dexamethasone (RD) has been an effective treatment for patients with relapsed or refractory Multiple Myeloma (RRMM). On the basis of the available evidence, treatment with RD may continue in responding patients until progression or unacceptable toxic effects. The data suggest full dose lenalidomide is important for optimal efficacy and to improve the progression free survival (PFS). Approaches to achieve higher doses of lenalidomide could include continuing therapy in responding patients and proactive adverse effects (AEs) management.

Aims: The main aim was to evaluate the incidence of two of most common non-hematologic AEs related to lenalidomide (rash and dystonia) in patients who received RD. The second end points were to evaluate the response of rash after switching the enoxaparin to bemiparin and to evaluate the response of the dystonia after treatment with clonazepam, instead of lenalidomide dose reduction.

Methods: We retrospectively reviewed a consecutive cohort of patients with RRMM receiving RD (R: 25 mg on days 1 through 21, d: 40 mg on days 1, 8, 15, and 22) in 28-day cycles until progression or unacceptable adverse effects, from 2011-2016. All patients received thombophrophylaxis with low-molecular weight-heparin (LMWH) (Enoxaparin 40 mg subcutaneous daily) the first 4 cycles; thereafter, patients were switched to aspirin 100 mg in a day prophylaxis. Clonazepam 7500 anti-Xa IU once-daily dose was employed if enoxaparin was suspended. Clonazepam dose to treat dystonia was 0.5 mg twice daily. Data were analyzed with SPSS statistical v 22.0.

Results: Between 2011 and 2016 a total of 65 patients received Rd in our center. Baseline characteristics are shown in Table 1. Patients received a median of 2 previous regimens (range 1-6). 51.5% of the patients had undergone one previous autologous stem-cell transplant (ASCT). Rash occurring in 12.3% of patients (grade 2), all of them were concurrently receiving enoxaparin. All rashes resolved switching the enoxaparin to bemiparin, maintaining same dose of lenalidomide. Neither treatment with esteroides or antidepressamin were administrated. Dystonias were reported in 23% of patients (grade 2), all of them disapperated after treatment with clonazepam without lenalidomide dose reduction.

Table 1.

Summary/Conclusions: Rash and dystonias are frequent adverse effects of immunomodulatory drugs (IMiDs), particularly lenalidomide, often leading to treatment discontinuation and decreasing the potential benefits to patients. According to our data, the rash could be due to synergism between enoxaparin and lenalidomide. In most cases, switch LMWH letting not to reduce lenalidomide dose in order to optimize the benefit of the treatment. Clonazepam, a benzodiazepine, is useful to treat dystonias related to lenalidomide.

PB1970

PROLONGED THROMBOPROPHYLAXIS IN PATIENTS TREATED WITH LENALIDOMIDE AND DEXAMETHASONE DOES NOT SEEM STRICTLY MANDATORY TO PREVENT LATE THROMBOTIC EVENTS

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Background: Risk of venous thromboembolism (VTE) in general population is 1% annually, significantly higher in oncologic setting, in particular with Multiple Myeloma (MM). Treatment with Lenalidomide plus Dexamethasone represents an additional risk factor for VTE, with most of VTE events observed in the first six months since therapy starting. No definitive data are available on the more appropriate duration of thromboprophylaxis (TP) in patients treated with lenalidomide.

Aims: To explore: I) the incidence of late thrombotic events in a real world population of relapsed MM, treated with Lenalidomide plus low dose Dexamethasone treatment (Len-dex), and concomitant TP with low molecular weight heparin (LMWH) performed for the first 4-6 months of therapy, without TP maintenance, II) the possible correlation between the presence of thrombotic risk factors and the occurrence of a late VTE.

Methods: We performed a retrospective analysis, after regular approval of local ethic committee, on chart data of 103 patients (pts) with relapsed MM treated with Len-dex according to label indication between January 2003 and December 2016 at our single centre institution. VTE prophylaxis was performed with daily dose of subcutaneous LMWH 4000 IU for 4-6 months, with no further TP, regardless of the presence of thrombotic risk factors.

Results: Main features of patients on study were: median age 66.3 years (range 41.9-85.2 years), median previous line of therapy 3 (range 1-7), time from diagnosis to lenalidomide starting 33.3 months (range 0.3-159.9 months), median duration of Lenalidomide treatment 8 months (range 0.4-65.2 months) with the following response: sPR 9%, 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 784 | haematologica | 2017; 102(2)
0.4–6 months). No hemorrhagic events were observed during LWMH. 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 diagnosis of MM to early VTE was 1 year (range 1-8.8 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 (≥PR 83%, 10 pts). Concomitant adverse events (AE) was registered 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, p=0.092).

Table 1. Baseline distribution of risk factors for thrombosis in the population on study.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; 65</td>
<td>10</td>
<td>83%</td>
</tr>
<tr>
<td>CVC/Perc贯彻落实</td>
<td>5</td>
<td>42%</td>
</tr>
<tr>
<td>Blood Transfusions</td>
<td>4</td>
<td>33%</td>
</tr>
<tr>
<td>Gene Mutation</td>
<td>2</td>
<td>17%</td>
</tr>
<tr>
<td>Paraprotein</td>
<td>3</td>
<td>25%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This study shows that LWMH 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 65 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 1.6-51.5]), 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), 10; 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, 49; VD, 21; VCD, 6), 6 patients received lenalidomide based therapy (Ld, 6), 10 patients were received MP therapy, 19 patients received dexamethasone therapy (high dose, 16; low dose, 3), 1 patient received radiation therapy as first line therapy, and 13 patients received only supportive care due to their fragility. After induction therapy, the overall response rate (at least partial response, PR) was 52.7% (stringent complete response (sCR) 0.3%, CR 4.5%, very good PR 16.1%, PR 29.5%). Overall survival (OS) was 74.5% at 1 year, 66.2% at 2 years with median follow-up of 19 months (range 1-52) for patients who were still alive at the date of last contact and 14 months (range 1-52) for entire cohort. Death occurred in 41 patients during the follow-up period. International staging system (ISS), with ISS1, 19; ISS2, 42; ISS3, 60; N/A, 4, can divide elderly patients into three distinct survival groups (P<0.001) (Figure 1A). Univariate and multivariate analysis showed a lower OS was associated with serum calcium level greater than 11 mg/dL (HR 3.036, 95%CI 1.412-6.529) (Figure 1B) and beta-2-microglobulin 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.

Summary/Conclusions: This study shows NDMM patients with bortezomib and lenalidomide-based induction therapy has a good outcome in the real world compared with the clinical trial.

PB1972
RETROSPECTIVE ANALYSIS OF 121 MULTIPLE MYELOMA PATIENTS USING THE R-ISS PROGNOSTIC STAGING SYSTEM AND RESPONSE TO FIRST LINE OF TREATMENT

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 treatment abroad. 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 4.28% of patients the actual date of diagnosis was before 2011 but 3.63% of patients after 2015. Median age of patients at presentation is 56 years old , 3.33% between30-40 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 into14% stage I, 31% stage II, 47% stage III. Restaging using the RISS 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 categorized in the third stage due to either high LDH level, high cytogenetic risk or
even both. First line treatment 55% of the patients received Bortezomib based triplet therapy, 22% received Ixo (Cytophosphamide, Thalidomide, Dexamethasone), 7% RD (Lenalidomide, Dexamethasone), 3% CyBord (Cytophosphamide, Bortezomib, Dexamethasone), 3% RV (Lenalidomide, Bortezomib), 2% Thal-Dex (Thalidomide, Dexamethasone), 2% RT (local Radiotherapy), 2% WatchfulWait, 1% MP (Melphan, Prednisone) and 3% refused for treatment and lost follow up.

Table 1.

<table>
<thead>
<tr>
<th>BSS stage</th>
<th>% of patients</th>
<th>BSS stage</th>
<th>% of patients</th>
</tr>
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<tbody>
<tr>
<td>Stage 0</td>
<td>15%</td>
<td>Stage II</td>
<td>30%</td>
</tr>
<tr>
<td>Stage I</td>
<td>31%</td>
<td>Stage III</td>
<td>20%</td>
</tr>
<tr>
<td>Stage IV</td>
<td>7%</td>
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</table>

Summary/Conclusions: Applying the RISS system to myeloma patients is a very effective and easy method to categorise myeloma patients, a significant number of patients in Kuwait are diagnosed as stage III, with median age of 56 years although the use of novel therapies shows excellent response to most of them.

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 (Rios-Tamayo et al, 2015) of multiple myeloma (MM) referenced to date. Here we update and point out the epidemiological variables of interest.

Aims: To highlight the importance of the epidemiological perspective in the knowledge and outcome of MM.

Methods: From January 1985 to February 2017 all consecutive patients diagnosed with MM at our institution have been registered, including clinical, biological and socio-demographic variables, as previously reported. A comprehensive approach to comorbidity was recorded as well as diagnostic and treatment delay. Overall survival (OS) was estimated by the Kaplan-Meier method.

Results: 700 patients have been included in the registry, 343 men (49%) and 357 women. All cases have their place of residence in the Granada province. The median age was 67 years (range: 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 (solar 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 IGM 55.6%, IGA 24.8%. Light chain only 15.9%, Non-secretory 3%, IGD 0.6% and IGM 0.2%. The International Staging System is known in 378 patients (25.9%), 27(10%), 34 (6.7%) and 36 (9.8%). Baseline performance status (ECOG) was: 0 (4.7%), 1 (41.1%), 2 (26.7%), 3 (21.7%), and 4 (5.9%). Comorbidity was assessed in 498 patients. 30.6% of patients were obese at the moment of diagnosis. 8.2% had other previously known or synchronous neoplasms. 150 patients (30.1%) had three or more comorbidities. The median diagnostic delay was 4.1 months (0.1-80) and median treatment delay was 13 days. 44 patients (6.3%) were very unwell and they did not receive active treatment. Information about stem cell transplant is available in 606 cases: 151 of them (24.9%) received a first autologous transplant. Median OS for the whole cohort was 43.1 and 24.4 months for patients younger than 65 years or 65 years or 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 neoplasia. Infection is the leading cause of death. Information derived from population-based registries may help to complement data from clinical trials.

PB1975

REAL WORLD USE OF IXAZOMIB WITH LENALIDOMIDE AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED AND RELAPSED REFRACTORY MULTIPLE MYELOMA

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Aims: To characterise real world use of IRD by demographics, response rate (RR) and progression free survival.

Methods: This was a retrospective review of patients sequentially treated with IRD at a large UK Haematology Centre. Patients received Ixa 4mg D1, 8, 15 and lenalidomide (dose as per label) days 1-21 and dexamethasone 40mg weekly or as tolerated every 28 days until disease progression or intolerance. In some cases, Ixa was added later to RD. RR and PFS were assessed according to IMWG criteria and haematological toxicities graded by CTCAE 4.0 criteria.

Results: Up to 31st October 2016, 30 patients were treated with the IRD schedule. Median age was 65 years (32-75), male (57%), ISS: stage I 18 (60%), stage II 4 (13%), stage III 8 (27%). 27 patients had a median of 2 (2-5) prior lines of therapy. All patients had previous treatment with a proteasome inhibitor (PI) (29 bortezomib, 5 carfilzomib) and 8 (27%) were refractory to a PI. 3 (10%) had prior lenalidomide and all remained sensitive. 23 (77%) had a prior autol-
ogous stem cell transplant. Out of those with results, 18 (69%) had adverse cytogenetics including 6 (23%) with TP53 loss. The median number of treatment cycles completed was 6 (2-35) with a median time on treatment currently of 5.5 months (1.6-40) for a median follow-up of 6.8 months (1.6-40). 24 patients were evaluable for efficacy analysis. 7 discontinued therapy, 6 due to disease progression of which 5 (83.3%) were refractory to a PI. 1 patient discontinued due to toxicity. The overall response rate (ORR) was 70.5% (95% CI 54.1%, VGPR 3 (12.5%), CR 1 (4.2%). For those refractory to prior PI, the ORR was 37.5% (PR 2 (25%), VGPR 1 (12.5%), 7 (29%) had Ixa added for sub-optimal response (<PR) or PD, if 2 had an improvement in response (VGPR and MR), 1 stabilised disease and the rest remained refractory. 5 (21%) had Ixa added whilst responding to RD, with the intention of deeper response and prolonging PFS. All continued to maintain their response. The median overall PFS was 19.23 months. The PFS for those refractory to prior PI was 11.6 months vs not reached for those sensitive (p=0.0159). Those with TP53 loss had a median PFS of 7.5 months. IRD was well tolerated with 5 (20.8%) patients withdrawing due to grade 3-4 neutropenia, thrombocytopenia and 1 patient experiencing grade 4 anaemia. This resulted in a 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 patient 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 LENALIDOMIDE AND POMALIDOMIDE IN RELAPSED/REFRACTORY MYELOMA PATIENTS IN A REAL WORLD STUDY

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Background: New agents have revolutionised the treatment of multiple myeloma. Immunomodulatory drugs (IMiD) such as lenalidomide and pomalidomide are now established in the management of relapsed/refractory myeloma. The aim of this study was to evaluate the efficacy and tolerability of lenalidomide and pomalidomide in real-world practice. The vast majority of patients had received prior thalidomide. The median number of previous treatment lines in those who progressed to pomalidomide was 12.8 (n=17), which is double that of the national average reported in seminal trials. These patients had to have an adequate response to lenalidomide, which was defined as stable disease (SD) or response to RD (partial response (PR) and very good partial response (VGPR)) in line with the largest phase 3 trial in the field. The median overall survival (OS) in the lenalidomide group was 13.6 months (10.1-17.1) and the pomalidomide group was 16.8 months (12.2-21.3).

Methods: We retrospectively analyzed the clinical data of 72 MM patients who received transplantation in the Hematology Department of the First People’s Hospital of Soochow University from May 2012 to June 2015. Among them, 36 patients underwent BUCY regimen while the others received high dose melphalan. Those were compared between the two groups including the complica-
tions of hematopoietic reconstitution and the post-transplantation efficacy.

Results: There were no significant differences in age, stage, induction therapy, mobilization method between the two groups. The transplantation-related adverse events were similar in both groups but the incidence of pulmonary infections 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 SCR/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%. Corre-

despondingly, 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 infer-
ior 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 comes 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 55.5 and 57.4 years. At first presentation nine had ISS 3, one ISS 2 and two ISS 1 stage disease, two patient presented origin-
ally as plasma cell leukaemia. FISH showed 1q amplification in 4, 13q deletion in 4, 17p deletion in 4, 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 SCR/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%. Corre-

despondingly, 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 infer-
ior to HDM regimen. In conclusion, BUCY regimen may replace HDM regimen as a standard conditioning regimen for ASCT in multiple myeloma.

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progression was 23.9 (3-65) months. Eight patients presented with cerebral nerve palsies, 2 with paraesthesia, 1 with hypertension 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, cranial-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).

Figure 1.

Summary/Conclusions: CNS progression in MM has a particularly poor prognosis as it represents a late stage of an aggressive relapse which often shows chemo-refractoriness. The differential diagnosis includes infection, autoimmune or vascular disease of the CNS as well as paraneoplasia and drug toxicity. The CNS penetration of the effective myeloma drugs is poor except for the imids, and drugs with CNS availability are usually not very effective in refractory MM.

PB1979

DARATUMUMAB: CHALLENGES OF INTEGRATING THIS NEW THERAPY INTO STANDARD CARE

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Background: Daratumumab (Darzalex) is the first anti-CD38 human Monoclonal Antibody approved for Multiple Myeloma (MM). Targeting the CD38 antigen on the surface of MM cells it causes apoptosis, and has an immune modulating 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; Obtaining RBC Tests for patients receiving Daratumumab: 95% 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).

Table 1.

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Number of prior treatments</th>
<th>Regimens</th>
<th>Disease outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>27-210-2016</td>
<td>59</td>
<td>2</td>
<td>1-2</td>
<td>IRRs</td>
</tr>
<tr>
<td>23-11-2016</td>
<td>59</td>
<td>2</td>
<td>1-2</td>
<td>IRRs</td>
</tr>
</tbody>
</table>

Infusion duration

Stage 45-59: 1-2 Infusion duration

Stage 59: 1 Infusion duration

Summary/Conclusions: Education, to include Blood Transfusion, Protein and Histopathology laboratory, and High Dependency Unit staff, in the key aspects of monitoring and risk management are an important part of integrating this new therapy to the treatment pathway for myeloma patients. Daratumumab is likely to become an important treatment for improving both Outcomes and Quality of Life for Myeloma patients going forward.

PB1980

MULTIPLE MYELOMA IN HIV+ PATIENTS LITERATURE REVIEW AND OWN CASE

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Background: Multiple myeloma (MM) and HIV infection in AIDS stage until now its considered not to be associated. 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 progression-free survival of HIV+ MM patients on HAART compared to HIV-negative MM patients appeared to be 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 ossealga and inabiility to move. Total protein 135 g/l with 81.7 g/l 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 1000 copies/ml, hepatitis C viral load 14.2 mln copies. He was started on HAART, combined with chemotherapy 5 courses of CP+V+MP+M and 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 Pegasys and lamivudine (Table 1).

Summary/Conclusions: Patients with MM and HIV infection did not differ significantly from the MM in HIV-negative with respect to age, gender, stages and renal function, and treatment with addition of HAART.Recently was reported that HAART itself may reduce and even remove m-gradient in HIV positive

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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>Characteristic of patients with HIV infection and MM</th>
<th>Age (mean ± SD)</th>
<th>%</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>45.2 ± 3.8</td>
<td>50</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>M-Gradient</td>
<td>0.4 ± 0.1</td>
<td>20</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Plasma cells/mm³</td>
<td>4.6 ± 0.8</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Bone lesions</td>
<td>1.2 ± 0.4</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>CRX</td>
<td>0.8 ± 0.3</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Platelets/mm³</td>
<td>200 ± 50</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>VAD/MAR/Melph (±Thal)</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>VAD/MAR</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Outcome</td>
<td>Death</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Response</td>
<td>Partial Response</td>
<td>30</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>
| Summary/Conclusions                                  | All 3 strategies for stem cell mobilization have their own merit. Steady state mobilization is safe and yields sufficient stem cells; however, patients require more apheresis sessions. Moreover, more than a quarter require additional therapy with plerixafor. Of concern, greater than half of these patients have increased myeloma markers during the interval between stopping chemotherapy and mobilization which may potentially affect outcomes. Mobilization with high dose cyclophosphamide yield more CD34+ cells but with increased toxicities - 50% of patients required admission for febrile episodes. Conversely, half of these patients had improvement in their myeloma markers. The use of low dose cyclophosphamide for mobilization resulted in lower admission rates (13%), however, plerixafor is required in a fraction. In light of these findings, we propose that patients who have not achieved at least VGPR should be mobilized with cyclophosphamide, the dosage dependent on their individual risks.

PB1982
MINIMAL RESIDUAL DISEASE MONITORING IN MULTIPLE MYELOMA PATIENTS BY FLOW CYTOMETRY: A SINGLE CENTER EXPERIENCE
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Background: Multiple myeloma (MM) is a malignant disease characterized by an increased number of clonal (abnormal) plasma cells in the bone marrow (BM). High-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (SCT) is used for the treatment of young MM patients and produces a high rate of complete remissions (CR). Recent trials with novel agent combinations alone have also resulted in high CR rates, even among old patients, high-risk patients and relapse/refractory MM. Unfortunately, most patients have a recurrences of the disease. This is due to the persistence of residual tumor cells, known as minimal residual disease (MRD), responsible for tumor relapse.

Aims: BM samples from 51 MM patients who had achieved partial or complete response or were resistant after chemotherapy, including autologous SCT, were evaluated by multiparameter flow cytometry (MFC). The study was conducted to assess the quality of remission, the correlation between the number of abnormal cells of BM and other signs of disease activity, readiness of patients for autologous SCT.

Methods: The study included 51 patients MM, average age - 54 years (36-70 years), who underwent assessment of MRD from November 2014 to February 2017. According to the classification Durie-Salmon the vast majority of patients (n=40) had III stage of disease, 8 patients – II and 2 patients – I. Response to treatment was assessed according to standard EBMT criteria. At the time of MRD assessment 20 patients were in CR, 8 had a partial response (PR) and 15 had a resistant disease; 5 had a primary MM, 3 patients were in the last line of treatment. Most of the patients were undergoing high-dose chemotherapy with autologous SCT (n=42). Re-evaluation of MRD after therapy was managed to hold in 36 patients at a mean of 3.1 months (1.9-5.7, min-max). Analysis was performed using a FACSCantoll flow cytometer (BD) and FACSDiva software (BD). Instrument performance was checked daily by recording fluorescence intensity with calibrating beads (Cytometer Setup and Tracking from BD Biosciences). Whole BM was estimated using combination of surface and intra-cellular staining CD38/CD56/CD27/CD117/CD81/CD19/CD45/cyLamba/CD138/cytKappa. The sensitivity of our panel MRD is 0.01% (i.e. 10^-4).

Results: Among patients in CR (n=20) confirmed the absence of MRD in 6 patients, but 14 CR patients were MRD positive. MRD was detected in all patients with PR and resistant disease (n=31). The relative content of abnormal plasma cells in CR patients with MRD positive (n=14) was significantly lower than that in PR/resistant patients (n=31): 0.00% (0.026-0.721%) versus 1.5% (0.203 -5.9%), p=0.0000092. PR patients (n=8) had a lower relative content of abnormal plasma cells (as expressed tendency), than patients with resistant disease (n=15): 0.286% (0.177-1.129%) versus 1.48% (0.90-8.0%), p=0.053. Besides the relative content of abnormal plasma cells in PR/resistant patients (n=31) correlated with the serum M-gradient level (r=0.42, p=0.019) and low dose plerixafor (r=0.54, p=0.0017).

Summary/Conclusions: Currently, we can conclude that MFC could be considered as the method of choice for MRD monitoring in MM. If the disease is measured, then, indeed, enough to evaluate only the M-gradient level of serum. If the M-gradient is not defined, it is necessary to assess the number of abnormal plasma cells in the BM and for the high-quality responses at the time of transplantation. And also it can help us to regulate duration of maintenance therapy.

PB1983
AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY MULTIPLE MYELOMA PATIENTS
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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 2017. Most patients received induction therapy including 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 5 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). PBSC were collected after high-dose cyclophosphamide (2 g/sqm in 2 pts, 3 g/sqm in 11 pts, 4 g/sqm in 22 pts) plus G-CSF, plerixafor was administered in 4 pts. Three pts also received lenalidomide and dexamethasone to improve the depth of response before ASCT. At the time of conditioning, among 34 evaluable pts, 8/34 pts were in complete response/ stringent complete response (CR/sCR), 19/34 in very good partial response (vGPR), 5/34 in partial response (PR) and 2/34 in stable disease (SD). The conditioning regimen consisted of melphalan 140 mg/sqm in 11 pts or 200 mg/sqm in 24 pts. A median number of 4.1 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 recovery after a median of 12 days (range 8-25) and platelets recovery after a median of 13 days (range 8-45) after transplant. No grade 3-4 toxicities were recorded. No transplant-related mortality was recorded within 100 days post transplantation. Three months after ASCT, among 28 evaluable pts, 10 pts were in VGPR and 14/28 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. MGUS is an asymptomatic, pre-malignant condition that can progress to MM. MGUS is a low-grade malignant disease with a risk of developing MM of 1-2% in 10 years of follow-up.

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 of 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 ≥2x10^9 or ≥20% plasma cells in the peripheral blood. It is an aggressive disease, with a median survival of 7 to 11 months. Due to its rarity, it is difficult to design prospective studies or randomized trials in PCL, so collecting and publishing data from the largest number of cases is essential for the understanding of PCL’s pathophysiology and outcome.

Aims: To characterize a series of PCL patients, in order to obtain data with the potential to be used as prognostic factors and to improve clinical outcomes.

Methods: Single-center, observational, retrospective study including all PCL cases admitted in our hospital between 2007 and 2016. Data regarding demography, clinical characteristics, laboratory results, treatment, follow-up and mortality were collected and analyzed using Statistical Package for Social Sciences (21st version), searching for significant associations (p<0.05) with overall survival (OS) and progression free survival (PFS).

Results: 15 patients were included, with a median age of 58 years. Most patients were male (60%) and had PS ECOG 0-1 (93.3%) at presentation and primary PCL (80%). Median hemoglobin (Hb) and platelet values were 8.5 g/dl and 74x10^9/L, respectively. Median plasma cell percentage was 37.3% (peripheral blood) and 60% (bone marrow). IgG heavy chain was present in 33.3% and lambda light chains in 53.3% of cases. Most patients had total serum calcium ≥4.5mmol/L (60%), total proteins ≥65g/L (86.7%), monoclonal component ≥30g/L (53.3%), albumin ≥35g/L (60%), creatinine clearance ≥50ml/min (66.7%), elevated β2-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%) had been submitted, at least, to an 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 ≤100x10^9/L, splenomegaly and lambda light chains. In multivariate analysis, only the presence of splenomegaly kept its association with OS; none of the characteristics associated with PFS kept their significance. Chemotherapy followed by ASCT and the achievement of, at least, vGPR after chemotherapy and ASCT were associated with longer OS and PFS.

Summary/Conclusions: This study’s retrospective design and the small sample size limit the strength of our data and our conclusions. Interesting results were obtained regarding pre-treatment prognostic characteristics and the association of improved OS and PFS with treatment response and ASCT execution. More studies are necessary to determine the clinical relevance of this findings and the best treatment strategies in PCL.

PB1986

OPTIMIZATION OF POMALIDOMIDE PLUS LOW DOSE DEXAMETHASONE IN REFRACTORY/RELAPSED MYELOMA MULTIPLE MYELOMA PATIENTS

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Background: MM-003 study has presented a median PFS of 4.0 months and median OS was 13.1 months overall for Pomaldoxim and low doses of dexamethasone in RRMM patients. Those results were better when a third drug was added (Poma-Dexa, Pom-Dexa-Cyclophosphamide-dexa, and Poma-Bortezomib-dexa, ORR 38.9, 64.7 and 85%; PFS 4.4, 9.5, 10.7 months respectively).

Aims: To evaluate the rate of treatment at therapy with pomalidomide plus dexamethasone in RRMM, and to analyze the efficacy of another drug in high risk MM.

Methods: We reported the clinical experience of the 8 patients treated with pomalidomide and dexamethasone. In patients with high risk MM (cytogenetic, extramedullary myeloma or plasmatic cell leukemia) pomalidomide and dexamethasone have been added to the 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...
pomal-bortezomib-dexa in 1 PCL patient. Table 1. Demographic characteristic’s patients. Figure 1. Response of monoclonal spike.

**PB1987**

**PROGNOSTIC SIGNIFICANCE OF PLASMA BLASTIC PLASMA CELLS IN THE ERA OF NOVEL AGENTS IN MULTIPLE MYELOMA**

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**Background:** Plasmablastic (PB) feature of plasma cells in multiple myeloma (MM) has long been identified as poor prognosis. Interestingly it does not take part of International Revised Scoring System (R-ISS). Similarly, the prognostic impact in the era of novel agents and novel classes in MM is unknown. Finally, the percentage of PB in the bone marrow to which a poor prognosis develop is unclear.

**Aims:** To assess which modality of treatment of plasmablastic MM was associated with longer progression free survival (PFS) and overall survival (OS).

**Methods:** We have performed a retrospective analysis of all MM in our center from May 2005 to November 2016, and sought for MM with plasmablastic features, characterized by immature cells with high proliferative index rate. The PFS and OS were calculated since the first time the PB morphology was observed in the bone marrow aspiration, in the outset at newly diagnosed patients or in relapsed patients.

**Results:** 65 patients with PB were included. Adverse cytogenetic per IMWG criteria was reported in 6 patients, del17p x3, (4;14) x3, and one with both. 33.8% were ISS 3, and 23.1% R-ISS 3. Extramedullary disease (EMD) was reported in 40%. 35 patients (53.8%) were in first-line therapy. The overall response rate with any triplet-based treatment containing always a proteasome inhibition and IMiDs or alkylator was 49.2%, with 29.2% VGPR and 4.6% CR. The median PFS and OS were 6.9 and 14.9 months as a whole, respectively. The median PFS was greater when treatment combined bortezomib, lenalidomide and dexamethasone: 36.1 months [0-99] vs 5.5 [0.59-10.4] otherwise: p=0.014. However, no difference in OS was demonstrated. Importantly, high dose therapy with ASCT was associated with longer PFS and OS, respectively 21.4 months [12.8-30.1] vs 2.83 [0-5.7] p=0.003 and 50.4 months [29.9-70.7] vs 6.27 [1.1-11.4] p=0.001. In multivariate analysis, poor OS was associated to acute renal failure at disease entry, presence of EMD, of del(17p), of hypercalcemia, and elevated lactate dehydrogenase.

In this pilot study between the AMR and ANZ MRDR demonstrates many similarities but also highlights significant differences, particularly in first line therapy and depth of response. Future studies between the AMR and MRDR will provide a platform for ongoing international benchmarking.

**PB1988**

**INTERNATIONAL OPPORTUNITIES TO COMPARE ‘REAL WORLD’ DATA FROM MYELOMA REGISTRIES: BASELINE CHARACTERISTICS, FIRST-LINE THERAPIES AND EARLY OUTCOMES FROM AUSTRIA AND AUSTRALIA/NZ**

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**Background:** Most outcome data for multiple myeloma (MM) come from clinical trials which can not necessarily be extrapolated to ‘real world’ patients. More information is needed on patients treated in the ‘real world’ and in a wider range of settings.

**Aims:** To compare and contrast baseline characteristics, investigations, and initial therapies in different geographical regions, Australia/New Zealand (ANZ) and Austria, through first analysis of data from two established MM registries on behalf of the steering committees of the Austrian 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 2001-2017.

**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 years: 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 67%/33%, respectively).

PRESENTATION: IgG myeloma was the most common subtype 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 monoclonal gammopathy was similar (m/f 21%/19% vs 21%/19% on the AMR and MRDR, respectively). INVESTIGATIONS: A higher proportion of patients underwent MRI (m/f 51%/58% vs 25%/27%) and skeletal survey (SS) (78% vs 60%) on diagnosis on the AMR than the MRDR, respectively. Baseline laboratory investigations were similar, however, patients on the MRDR demonstrated higher median LDH (m/f 176:178 vs 187:186 units/L) and serum calcium (m/f 2.34:2.28 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 (ECOG 1 m/f 43%/44% vs 81%/78%, on the AMR and MRDR, respectively).

FIRST LINE THERAPY: First line therapy was predominantly bortezomib (Velcade - V) based on both registries (81% vs 85%). Dexamethasone (D) was the most common on the AMR (29%) followed by V/thalidomide/D (VTD) (25%) with VCD (35g/L) most common 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.

**PB1989**

**DETECTING EARLY RELAPSE IN MULTIPLE MYELOMA AFTER ASCT: USEFULNESS OF IMMUNEASSAYS**

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**Background:** The Free Light chain immunoassay (FLC) (Bindingsite, Birmingham, UK) is part of the mandatory response assessment for MM, the role of the Heavy/Light Chain immunoassay (HLC) is under investigation. Also relapses in MM patients are frequent, autologous stem cell transplantation (ASCT) is the standard consolidation therapy and there is an interest to detect early relapses in the consolidation therapy. We hypothesized that the combination of these techniques could permit to detect early biological (non-symptomatic) relapses (EBR) in this setting.

**Aims:** To analyze the usefulness of HLC and FLC to detect EBR in MM after ASCT.

**Methods:** Retrospective study was performed following these criteria: all patients diagnosed of secretory MM, in our center, and treated (including ASCT), between May 2011-August 2015; the protocol for follow-up included FLC, HLC, serum and urine electrophoresis (SPE, UPE) with immuno fixation (IFX), pre-ASCT, after 12
weeks and every 3 months later (minimum follow-up: 6 months). EBR was defined as 25% on p-creatinine increase (any amount for patients on CR/SR) and/or ≥20mg/dl FLC increase, and/or 25% involved FLC increase with abnormal ratios. For urine, an increase >500mg/24 hrs of involved free-chain protein.

Results: Fifty-five patients were registered. Median follow-up 47 months. EBR rate: 25% (22/89), p-creatinine increase 30.0% (26/89), FLC increase only 20.2% (18/89). Median time to EBR was 8.5 months (range: 0.5-24.0). Treatment was determined by the physician and in all cases included a new agent. Fifty-five percent (49/89) patients presented an infectious complication and 54.7% (48/89) had abnormalities in other systems. Twenty patients (22.4%) had an infectious complication and a new agent regimen. In the period 2004-2010, patients were treated with an infectious complication and a new agent regimen (17.5% vs 28.1%), P=0.03. Twenty patients had infectious complications and new agent therapy compared with no treatment (17.5% vs 28.1%); P=0.03.

Discussion: New agent therapy for patients presenting an infectious complication and a new agent regimen in the first 3 months after diagnosis significantly improved the risk of EBR (17.5% vs 28.1%, P=0.03). The results of this study indicate that new agent therapy may be effective in reducing the risk of EBR in patients with newly diagnosed multiple myeloma. New agent therapy should be considered as a potential treatment option for patients presenting with infectious complications within the first 3 months after diagnosis.

Figure 1: A large graphic; legends: red: pre2013; blue: post2013.

Summary/Conclusions: Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potential "modifiable" variables and acting on them improves the short-term prognosis of patients with NDMM like: Supportive treatment to prevent infectious complications (avoid unnecessary hospitalization, antibioprophylaxis) and rapid access to optimal antiMM treatments. These improvements of short-term
Background: Multiple Myeloma (MM) is mainly a disease of the elderly and the very elderly patients (80 years of age or more) comprise one third about of all MM patients. This subset of patients suffer from concomitant disabilities and/or comorbidities and require a different and more individualized therapeutic approach, including the novel agents.

Aims: The aim of our study is to verify safety and efficacy of novel agents with the reliability to maintain a good quality of life and obtain a maximal disease control.

Methods: Patients from 8 Hematology Centers of the “Rete Ematologica Pugliese (REP)” were included in this study. Between January 2011 and December 2016, 71 patients (MF: 42/29) with a median age of 82 years (range 80-91) were diagnosed as newly symptomatic MM. Of the entire study population, 40 (56%) patients showed an ECOG score lower than 2. According to immunoglobulin heavy and light chain isotypes, patients had IgG-k (n=23), IgG-l (n=16), IgA-k (n=14), IgA-l (n=6), micromolecular k (n=8) and λ (n=4) chains. On the basis of ISS, patients were classified as I (n=4) score, II (n=23) and III (n=44) score, respectively. When CRAB features were considered, bone lesions represented the most frequent (n=43, 60.6%) clinical manifestations, while anemia, hypercalcemia and renal failure were found in 35 (49.3%) (2.8%) and 2 (2.8%) patients, respectively. Majority of patients (n=49, 69%) showed at least 1 comorbidity requiring specific treatments, and 11 patients (15.5%) showed more than 3 comorbidities. Patients were treated according to Bortezomib-based regimens (VMP, VCD and VD) (n=45; 63.4%), Lenalidomide-based regimen (n=4; 5.6%), and Thalidomide-based regimen (MPT) (n=5; 7%). Only 13 patients (18.3%) did not receive any novel agent.

Results: Based on IMWG criteria, 15 patients (21.1%) achieved a CR, 15 patients (21.1%) a VGPR and 15 patients (21.1%) a PR. Fourteen patients (19.7%) and 12 (17%) patients experienced a SD and a PD, respectively. As second line of treatment, Bortezomib was used in 14 (33.3%) patients, Lenalidomide in 17 (40.5%) patients and Thalidomide in 3 (7.2%) patients. Height patients (19%) were treated with old drugs (Melphalan, Cyclophosphamide or Bendamustine). Pomalidomide was used as third line-therapy in 3 patients. After 72 months (median 32.5 months) of follow-up, 33 (46.5%) patients remained alive with a median survival of 32.5 months and 25 (28.2%) died. Last follow-up from 13 patients was unavailable. Hematological and extra-hematological toxicities were similarly distributed (18.3% and 18.3%, respectively) and usually weak/moderate. Neuropathy was the most common toxicity reported (n=7, 9%). Patients treated with only novel agents (n=58), hematological and extra-hematological toxicity was observed in 14% and 16% patients, respectively.

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.
Summary/Conclusions: Multiple myeloma patients with concurrent HIV infec-
tion that is controlled on HAART tolerate ASCT for treatment of myeloma as
well as myeloma patients without HIV infection and have generally good out-
comes.

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 prolifer-
ation 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 44 MBq of FDG. Images were analyzed using an iterative thresholding algo-
rithm that delineates a continuous region based on Hounsfield units from the CT data (OsiX software; Pixmeo SARL; Bernex, Switzerland), allowing for seg-
mentation of the total skeleton on a fused PET/CT image. This enabled the quantifica-
tion of FDG uptake representing the entire skeleton, providing a global sum of uptake that considers all bone marrow involvement. Global SUVmean scores were compared before and at EOT using a two-tailed paired t test.

Results: A decrease in marrow FDG uptake was observed at EOT compared to baseline in most patients. The calculated global SUV/mean uptake decreased after initiation of treatment in 17 (73.9%) of the cases and increased in 6 (26.1%) of the cases. The changes were analyzed at baseline, all patients pro-
ceed to mobilization therapy cyclophosphamide 3g/m2 and received pegfil-
ulated growth factor 200mcg/m2 subcutaneous 7 days after chemotherapy. The median time to next treatment (TNT), defined as time from transplant to next new ther-
tapy or death of any cause.

Summary/Conclusions: 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 dis-

PB1996
VALUE OF MYELOGRAM PROGNOSTIC INDICIES 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 transplan-
tation in myeloma treatment as well as prognostic indices in era of novel drugs.

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 therapy. All patients provided informed consent so they may be translated into risk adapted therapy approach.

Results: A total of 152 biopsies were performed during the study period: see Table 1.

Summary/Conclusions: Prognosis in AL amyloidosis is slowly improving with the use of new anti-myeloma drugs and may improve further with new mono-
clonal antibodies. Therapy requires an early and accurate diagnosis. We do not perform random biopsies of tissues such as fat or gingiva due to low sen-
sitivity. In contrast, our hands biopsy to diagnose AL amyloidosis of what is usually published. Biopsies of clinically involved organs yields almost 100% sensitivity. Random biopsies of gingiva, subcutaneous fat 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%) 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: 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 1-25 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 markers and 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 fluorescent in situ hybridization, were found in 2 and 4 patients, respectively. The median initial dosage of Len was 15 mg and DEX 20 mg. The overall response rates were 68.6% and the 2-year progression-free survival was 70.8% at a median follow-up of 26.5 month. Grade 3 to 4 nonhematologic AEs were observed only in 8 patients. We estimated the AUC0–24 of Len by using formula as we previously reported (Ther Drug Monit 2014) and the cut-off value of the hematologic AEs was 2973 Sng/hr/ml (sensitivity 81%, specificity 80%) and non-hematologic AEs 2032 Sng/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).

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 1-25 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 markers and 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 fluorescent in situ hybridization, were found in 2 and 4 patients, respectively. The median initial dosage of Len was 15 mg and DEX 20 mg. The overall response rates were 68.6% and the 2-year progression-free survival was 70.8% at a median follow-up of 26.5 month. Grade 3 to 4 nonhematologic AEs were observed only in 8 patients. We estimated the AUC0–24 of Len by using formula as we previously reported (Ther Drug Monit 2014) and the cut-off value of the hematologic AEs was 2973 Sng/hr/ml (sensitivity 81%, specificity 80%) and non-hematologic AEs 2032 Sng/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).

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.
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, Figure 1). Expression of CD56 on the surface of extramedullary MM cells was found in 76,5% patients. OS in the group of patients with CD56+ 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+ 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, 1Hematology, Ematologia e trapianto/au federico ii, 2Hematology, AORN Carizzo, 3Scientific medical-laboratory center, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine

Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in newly diagnosed Multiple Myeloma (MM). Aims: It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rMM), whose prognosis is particularly severe. A regional prospective real-life analysis of patients with rMM who had been treated with BVD as salvage therapy has been performed.

Methods: 56 patients (31 M/25 F, Table 1), with rMM, median age at diagnosis 57.3 years (range 37-83), median age at start of treatment 61.8 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1.4,8,11. Dexamethasone 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim days +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 12 patients, in particular one del13q and one (11,14). All the patients had previously been treated with schedule containing bortezomib and IMiDs, and 30% had also received radiotherapy. 67% of them had undergone at least to a single autSCT. All patients were relapsed and refractory to last therapies received in SD, which can be considered as an impressive result in this subset of rMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second autSCT, and for two patients a bridge to alloSCT. Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (range 6-151), median OS from start of Bendamustine was 9.8 months (range 2-36).

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 B. Samir1,2,*, Y. Kolesyński3, V. Syvolapi1, A. Abramov3 1Department of Hematology, Zaporizhzhia Regional Clinical Hospital, 2Department of Internal diseases, 3Department of Pathophysiology, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine

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 microvessels density in multiple myeloma and parameters of disease progression. Endothelial cells participate in inflammatory events leading to apoptosis by regulating entry via the endothelial barrier. It is evaluable in 12 patients, as a transmembrane protein probably 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.

Aims: We 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, 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 circulantes 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 disease progression risk was 1.31 ng/ml with AUC value 0.836 (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=16.11-71) months vs 7.35 (IC=5.75-8.95) months (p=0.02).

Summary/Conclusions: The serum VE-cadherin level is a valuable biomarker for predicting treatment response and an independent prognostic factor for progression-free survival for patients with multiple myeloma.

PB2003

THE UTILITY OF FACS PURIFICATION OF PLASMA CELLS FOR FISH ANALYSIS IN MONOCLONAL GAMMAPATHIES


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Background: Despite the prognostic value of chromosomal aberrations, conventional metaphase karyotyping in monoclonal 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 – such as the PC in MG – reducing the confounding noise. This is particularly relevant when the percentage of pathologic cells in the sample is low, such as in monoclonal gammapathy of undetermined significance (MGUS) where, by definition, there are less than 10% PC in the bone marrow.

Aims: This study aims to analyze the utility and effectiveness of FACS purification of PC for the cytogenetic workup of MG by FISH.

Methods: We analyzed all FISH studies performed in our laboratory, in individual patients, on clonal interphase FACS separated bone marrow PC, between the 1st June 2015 and the 15th September 2016. The probes used in our standard MG panel were del(1p32), 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 apply a confident and adequate 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 3932270 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 67.6% of samples; 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 multiple myeloma (MM), with an identical median age (64.2±6.9 vs 63.0±10.8 years old, p=NS). We found that 16.3% (of 92) were positive for t(4;14), 12.2% (of 90) for del(17p13.1), 5.6% (of 90) for del(1p32) and 41.1% (of 90) for amp(1q21); t(14;16) was not identified in any of the 30 patients in whom the probe was used. The t(4;14) translocation was present in 22.4% of MM and 7.7% of MGUS patients (p=0.055), and del(17p13.1) was found in 18.5% vs 2.8% of MM vs MGUS patients (p=0.001). The del(1p32) positive results were identified in both MM and MGUS patients (5.6% vs 5.6%), p=NS and amp(1q21) (46.3% vs 33.3%, p=NS) were identified by all 5 probes in the MGUS patients (80% were positive for over 50% of the aberrations). We observed that 40.4% of MM and 65.8% of MGUS patients were positive for 20% of less of the tested aberrations, while 54.4% vs 34.2% were positive for 20 to 50%, and 5.3% vs 0% were positive for over 50% of the aberrations. In MMUS, 41.1% were positive for at least 80% of the aberrations. In MMUS, 41.1% were positive for at least 80% of the aberrations.

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 8-fold higher than the number we consider invariably sufficient to apply 5 probes, which we achieved in at least 80% of patients.
Summary/Conclusions: Although both PPN and CIDP patients suffer from sensorimotor symptoms, CIDP patients were more often associated with superimposed motor symptoms. Among PPN patients, demyelinating type neuropathy seems to be associated with more severe clinical presentations. Worsening of neuropathic symptoms in PPN patients warrants a high level of suspicion of malignant transformation of underlying disease.

PB2005

MOLECULAR GENETIC CRITERIA PREDICTING THE EFFICIENCY OF PERIPHERAL BLOOD HEMATOPOIETIC STEM CELLS TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Global gains in treatment of MM using auto-PBHSCT testify to heterogeneity of long-term outcomes of transplantation - different term of the achievement and duration of complete remission, progression-free survival (PFS), overall survival (OS). These facts determine individual approach to the approach and timing of PBHSCT.

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 with 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 patient's treatment is essential. It was determined that the carriage of the allele HLA-DRB1*03: 02 in MM patients is associated with a high risk of high-dose chemotherapy resistance (F=4.83, p=0.028; OR=1.75, p=0.038), and achieving remission after auto-PBHSCT is associated with carriage of haplotype HLA-C*06:02 - HLA-DQA1*01:01 (F=4.87, p=0.028; OR=1.74, p=0.038). Abnormalities of chromosomes 4, 11, 13, 14 and 17 were determined in 35 of 61 (57%) MM patients with complicated disease course and minimal therapy response. Significant alterations were revealed in the presence of two or more abnormal clones (23 patients (37.7%), Ro Spirman=0,42, p < 0,05), deletion of chromosome 17 (17 patients (27.9%), Ro Spirman=0,41, p < 0,05), deletion/monosomy of chromosome 13 (10 of 15 patients surveyed, Ro Spirman=0,33, p <0,05), the translocation t(4;14) (4 patients (6.6%), Ro Spirman=0,50, p <0,02).

Summary/Conclusions: The results indicate the necessity of introducing the molecular genetic support into protocol of examination MM patients on various stages of treatment with auto-PBHSCT.

PB2006

THE INFLUENCE OF MINIMAL RESIDUAL DISEASE AND TUMOR LOAD ON THE PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA PATIENTS

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Background: Use of modern drugs and their combinations in the complex antmyeloma therapy (induction, high-dose therapy (HDT) with autologous stem cell transplantation (ASCT), consolidation and maintenance therapy) to improve efficacy of treatment and duration of responses. Despite the achievement of complete response (CR) many patients has a relapse which is caused by activation of residual clonal plasma cells.

Aims: To define influence of induction therapy regimens, HDT with ASCT to the frequency of Minimal Residual Disease (MRD) negative status and estimate a role MRD in duration of Progression Free Survival (PFS) in multiple myeloma (MM) patients.

Methods: We analyzed 52 patients with MM (median age 55 years, male/female – 2:1). The induction therapy with Bortezomib-based regimens (VDD, OD, VMP, PAD) was used in 36/52 (69%) patients, Immunomodulator-based regimens (Thal+D, OD, RD, VRD, PomD) – in 14/52 (27%), chemotherapy – in 2/52 (4%). ASCT is carried out 31 (59.6%) patients. Primary tumor cells phenotype and MRD were detected by 5-color flow cytometry. Clonal plasmatic cells were detected by markers: CD38, CD138, CD45, CD19, CD20, CD27, CD38+CD138+CD45- cells. MRD-negative status considered in identifying less than 1 tumor cell in 10000 (0.01%).

Results: MRD-negative CR was reached in 23.8% (10/42) patients after 4-6 cycles of therapy. The frequency of MRD-negative status in the “Bortezomib group” was 31% (8/26), in the “Immunomodulator group” – 7.7% (1/13) (Chi-square =0,1; p > 0,05). The general frequency of MRD-negative CR after HDT with ASCT was 33.3% (7/21). The carrying out HDT with ASCT allowed to MRD eradication in 36.4% (4/11) patients. One patient with a “light chain” myeloma lost MRD-negative CR after HDT with ASCT that led to development of a clinical relapse after 6 months. Carrying out a maintenance therapy with bortezomib or lenalidomide didn’t allow to achieve MRD-negative status in patients with MRD-positive response. On the contrary, achieve ment MRD-negative status promoted to increase of PFS. The PFS median in MRD-positive group of patients (n=36: 21 CR, 6 VGPR, 9 PR) was 21 months, in the MRD-negative group (n=16) – 66 months (p=0,0009) (Figure 1).

PB2007

QUALITY OF RESPONSE AS PREDICTOR OF SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN REAL LIFE MULTIPLE MYELOMA PATIENTS IN A SINGLE INSTITUTION

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is the standard treatment approach for younger patients with multiple myeloma (MM). Since the introduction of proteasome inhibitors and immunomodulatory drugs in MM treatment more patients achieve deeper and durable responses and better disease control before ASCT.

Aims: To evaluate the association between the depth of response before ASCT and survival outcomes in a cohort of patients with MM.

Methods: Retrospective analysis of patients with MM treated with HDC and ASCT between 2007 and 2016 in a single institution. All patients received high dose stem cell support after conditioning with high dose melphalan (200 mg/m2 and 140mg/m2 for patients with renal insufficiency). Response was assessed 100 days after ASCT according to the International Myeloma Working Group response criteria. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between groups was performed with log-rank test. The prognostic factors of survival were analyzed by Cox regression univariate and multivariate analysis.

Results: We included 195 MM patients, mainly males (57.9%) with a median age at ASCT of 61 years (28-71). The most prevalent subtype was IgG k (44.1%). The median number of previous therapeutic lines was 1 (1-4) and the majority of patients (61%) received bortezomib as part of first-line regimen. Patients undergone ASCT within a median of 10 months after diagnosis. With a median follow-up time from ASCT of 28.55 months (2.8-121.4), OS at 2 and
Five (29.4%) patients presented with leptomeningeal infiltration; in three of them it was diagnosed at the time of the diagnosis of PCL. All the patients had neurological features. Thirteen (76.4%) patients were able to start a curative treatment: VD in 7 (53.8%) patients, VTD in 2 (15.4%), VAD in 1 (7.7%), D-PACE in 1, M-TX-ARAC in 1 patient and RD in the remaining one. Three patients received intrathecal treatment. The intention-to-treat response was: 2 (15.4%) CR, 2 PR, 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% 0.47-4.76) months and median of OS was 4 (IC 95% 0.47-7.53 months).

PB2009

MANAGEMENT AND OUTCOMES OF PATIENTS WITH MULTIPLE MYELOMA IN REAL-WORLD SETTINGS IN BULGARIA, CROATIA AND SLOVAKIA

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Background: The multiple myeloma (MM) treatment (Tx) landscape is rapidly evolving, with varying Tx practice patterns and access schemes across countries. However real-world (RW) data describing patient (pt) management, MM Tx use and outcomes in some Eastern European Countries are limited. Aim: To understand the characteristics, management, Tx patterns and outcomes of pts with symptomatic MM in a RW setting in Bulgaria (BG), Croatia (HR) and Slovakia (SK).

Methods: Data were collected within a cross-sectional (X) and retrospective (R) phase of a chart review in 6 countries between June/15 and June/16 by (onco-)hematologists who managed at least 15 pts with MM per month (mo) and were responsible for initiating MM Tx. Data from 3 countries with limited access to MM Tx are shown. In the X-phase, data included characteristics and current Tx by line of therapy for all pts with MM seen during a 3-week observation period, regardless of pts' Tx status and strategy. In the R-phase, data included pt and disease characteristics at diagnosis, Tx response, comorbidities and outcomes by Tx line. Pts were selected in reverse chronological order and those who had completed specific lines of active Tx within the past 3 mo were included as follows: 3 pts in first line (1L), 4 pts in second-line (2L) and 7 pts in third or higher lines. Analyses were descriptive.

Results: In the X-phase, 7 physicians from BG, 6 from HR and 5 from SK included 43, 39 and 44 pts respectively. In the R-phase, 7 physicians from BG, 6 from HR and 5 from SK included pt and disease characteristics at diagnosis,Tx response, comorbidities and outcomes by Tx line. Pts were selected in reverse chronological order and those who had completed specific lines of active Tx within the past 3 mo were included as follows: 3 pts in first line (1L), 4 pts in second-line (2L) and 7 pts in third or higher lines. Analyses were descriptive.

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, metotrexate and dexamethasone is not today a standard of care for patients with PCL.
LENALIDOME AT THE DOSE OF TWENTY-FIVE MG EVERY OTHER DAY

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 (68%) 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 suboclusion, 1 mucositis); 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). Median overall survival was 11 months (range, 2 – 37). 10 of 12 patients relapsed after a median time of 5 months (range: 2-12). Concerning two patients not relapsed, 1 patient died in partial response 8 months after therapy of other causes; 1 patient is still alive, in complete remission 18 months after melphalan. He underwent ASCT and maintenance with lenalidomide.

Summary/Conclusions: Many patients refractory to proteasome inhibitors and IMIDs are probably still sensitive to alkylating agents and could be rescued with medium dose melphalan. We suggest therefore melphalan as a “bridge” strategy for further therapy, particularly in patients needing immediate disease control. Even in this era in which several novel drugs became available, single shot medium dose melphalan could be an affordable and safe therapy, able to achieve remission and to reduce disease burden prior to targeted therapy.

PB2011
LENALIDOMIDE AT THE DOSE OF TWENTY-FIVE MG EVERY OTHER DAY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND RENAL FAILURE: A REAL-LIFE EXPERIENCE
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Background: Lenalidomide, available as oral compound, is an IMID with both antiproliferative and immunomodulatory activity which is largely used in the management of newly diagnosed, relapsed or refractory MM and as maintenance therapy after autologous stem-cell transplantation. Due to its renal route of excretion, it is mandatory to adjust lenalidomide dose in patients with RI, guided by Creatinine Clearance (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 its clearance is not significant. However, there is no theoretical assumption against the possibility that protracting the time of full standard doses can be equally effective and tolerated by patients 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 51.5ml/min, range 20-148). Median progression free survival was 11 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 tempetime 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 CR, with or without concurrent diseases. Primarily, we performed complete blood analysis, with eGFR (CKD-EPI and MDRD methods), serum and urine electrolytes, bicarbonatemia, serum and urine immuno- fixation, fraction 3 and 4 of complement, cilioglobulimen, HbA1c, arterial gas analysis, evaluation of urine rate every 6 hours, daily urine collection, urine sediment. We also collected anamnesis on eventual nephrotoxic concurrent therapies like ASA, FANS, clinical parameters and objectives signs of RI (edema, symptomatic disonia). On the second day of hospitalization we requested protein electrophoresis on serum and urine, chest X-ray, ultrasonography of abdomen, echocardiography and electrocardiography. On the day three we evaluated creatinuria, sediment, serum ferritin and we decided, if necessary, to perform IVU investigation (bone marrow in suspected unknown MM pts, renal in suspected CN pts, umbilical fat for amyloidosis). All analyses were daily and colleaguesy 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 knewed 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 Recommandations in a routine clinical practice confirmed its feasibility and utility in the optimal workup of MM pts. We obtained diagnosis of RI within 4 days, both in known and in de novo MM pts, with a positive impact on reduced hospitalization, unnecessary dialysis and steroids overtreatment.

PB2014

NOCARDIOSIS PROVOKED BY NOVEL AGENTS AT RELAPSED MULTIPLE MYELOMA: CASE SERIES

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Background: The proteasome inhibitors and immunomodulatory drugs which are used in MM treatment enhance the risk of infection by several mechanisms.Nocardial infections are rare in Turkey.

Aims: Here, we present three relapsed myeloma cases which developed nocardia pneumonia.

Methods: Case-1: 66 year old man, who has a history of autologous SCT 4 years ago and lenalidomide usage because of IgG kappa type myeloma, has been prescribed bortezomib for the relief 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, with his lymphocyte count was 1200/mm3 and flow-cytometric analysis couldn't be performed. Thorax CT showed 39x39x45 mm mass like lesion. Broncoscopic lavage showed branchial bacillus via modified acid-fast and Gram stain. This typical morphological appearance was defined as Nocardia spp. The 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 therapy. 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 under the treatment of lenalidomide and dexamethasone. Her lymphocyte count was 1290/mm3 and flow-cytometric analysis showed CD5: %68 and CD20: %2. Thorax CT showed 39x39x45 mm mass like lesion. Broncoscopic lavage showed branchial bacillus via modified acid-fast and Gram stain. This typical morphological appearance was defined as Nocardia spp. The 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 therapy. Case-3:72 year old man, who has a diagnosis of IgG kappa type myeloma from broncoscopic specimen analysis, so imipenem-cilastatin therapy has been prescribed for the relapse of the disease. His lymphocyte count was 2300/mm3 and flow-cytometric analysis showed CD5: %68 and CD20: %2. Thorax CT showed 4 cm sized cavity and sputum microscopy showed 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 therapy.

Figure 1.

Table 1.

<table>
<thead>
<tr>
<th>Myeloma Type</th>
<th>IG kappa</th>
<th>Autoimmune</th>
<th>Hyperviscosity</th>
<th>Renal Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>66</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Lungopores</td>
<td>1290</td>
<td>2437</td>
<td>2437</td>
<td>2437</td>
</tr>
<tr>
<td>Hospital</td>
<td>328</td>
<td>328</td>
<td>328</td>
<td>328</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td></td>
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</tbody>
</table>

Results: See Table 1 and Figure 1.
PB2015

STUDY USE OF 18-F FDG PET/CT SCANNING INTO THE FIRST FOLLOW UP OF PATIENTS WITH MULTIPLE MYELOMA AND ASSOCIATION WITH BIOCHEMICAL RESPONSE

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Background: Positron computed tomography (PET/CT) with 18F fluorodeoxyglucose-labeled glucose (FDG) is a reliable technique with high sensitivity and specificity for assessing skeletal involvement and recent studies propose it as a method for predicting treatment response in multiple myeloma. Conventionally, the response is measurable by the monoclonal component in both serum and urine and Minimal residual disease (MRD) by flow cytometry has been established as a mandatory tool. The studies are aimed at combining the measurement of paraprotein with imaging tests that help to promptly define response or failure to the treatment.

Aims: The primary endpoint was the correlation of the biochemical response with the FDG PET/CT in a second evaluation after first line treatment. The secondary endpoint was the correlation between MRD and with second FDF PET/CT.

Methods: We included in this retrospective and observational study at Universitario 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 hypercalcaemia and 66% showed immunorepores. 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 a extramedullary plasmocytoma and nine had an anormal ratio of light chains. Seventeen patients were treated with bortezomib-based regimens, (median 5.5 cycles) included VTD, MPV, VLD and VD. After treatment, fourteen patients achieved complete response, two partial response and two had progressive disease. PET/CT was positive in all patients pretreatment, 15 focal lesions, 2 diffuse bone marrow involvement plus focal lesions and 1 involvement of bone marrow alone. Twelve patients had more than 3 focal lesions and two had extramedullary disease. At the end of first line treatment, PET/CT was negative in eight patients (44%) and fourteen had complete biochemical response (78%). 62% of the patients with negative PET/CT showed negative flow minimal residual disease (MRD) and biochemical complete response. Two patients had PET/CT with progression disease and corresponded to a biochemical progression.

Summary/Conclusions: The correlation between PET/CT and biochemical response obtained after treatment is 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 these findings. PET/CT negative is an image technique that could be a tool to follow up patients after the first line treatment added to the evaluation of the biochemical response.

PB2016

MULTIPLE MYELMA IN BORNEO SARAWAK: A DEVELOPING WORLD’S EXPERIENCE

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Background: Sarawak, is the largest state of Malaysia situated on the island of Borneo. Sarawak General Hospital is the tertiary referral center of Sarawak (serving a population about 1 million people). It is 980 km away from its main hematoly/transplant referral center in Kuala Lumpur, Malaysia, which is accessible only by airplane. Hence, treatment of patients with multiple myeloma in this part of the state is a big challenge due to its geographical constraint.

Aims: To identify demographics and clinical characteristics of patients with multiple myeloma; To establish treatment and outcome of patients with multiple myeloma.

Methods: This is a retrospective study examining basic characteristics and clinical outcomes of patients diagnosed with multiple myeloma between 2010 and 2016 in Sarawak General Hospital. Patients’ case notes 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 hypercalcaemia (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 survival rate was comparatively lower to our counterparts. Limitations wise, due to logistic and economic reasons, we do not have good access to cytogenetic and genetic profiling that enables us to prognosticate patients accordingly.

PB2017

A RETROSPECTIVE AND PROSPECTIVE AUDIT OF RADILOGICAL INVESTIGATIONS FOR SUSPECTED CASES OF PLASMA CELL DYSCRASIAS/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 (2016) 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 imaging for myeloma to assess for bone disease or EM plasmacytoma.

Methods: This retrospective and prospective audit included all patients having a skeletal survey. The biochemical response was defined according to the standard IMWG response criteria.

Results: The indications for requesting imaging is shown in Table 1A. No WB MRI/CT was performed in this period. 26% patients had new lytic lesions on skeletal survey. 23 patients had further imaging in the form of MRI or CT following skeletal surveys. All the positive MRI findings offered additional diagnostic information - including examples of missed multiple spinal deposits. The results of imaging are summarised in Table 1B. The false negative rate for skeletal surveys was 39% and the false positive rate was 22%. Following NICE guidance publication 23 patients had skeletal surveys performed for suspicion of myeloma between 10/2/16 and 30/5/16. The indications are summarised in Table 1C. No WB imaging was performed. 5 patients had positive skeletal surveys. 6 patients had subsequent CT/MRI imaging. A skeletal survey was report normal with a subsequent MRI showing multiple spinal deposits. The imaging results are summarised in Table 1D.

Table 1.
economic model for imaging with WB MRI. In addition it reviews evidence which links time to diagnosis to survival and myeloma related complications. The NICE guidance offers clear evidence that WB-MRI should be the investigation modality of choice for suspected myelomatous disease. It offers a diagnostic and cost-effective strategy that will ensure health improvements for myeloma patients. This audit offers further evidence of the diagnostic accuracy of MRI imaging. At present failure to comply with NICE guidance will lead to delayed diagnosis of myeloma in certain patients and potential patient harm. Therefore I offer a business and health improvement case for the Western Trust to instigate WB-MRI imaging for all suspected myelomatous bony disease.

PB2018
TONI DEBRE FANCONI SYNDROME DURING MYELOMA, ABOUT 8 CASES
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Background: The cast nephropathy with cylinders is the most frequent renal complication of the myeloma, which results from a catabolism of the light chains by the tubular cells and can lead a tubular chronic suffering showing itself by a syndrome of acquired Toni-Debré-Fanconi marked by a glycosuria, a phosphaturia, an aminoaciduria, a sometimes severe and sometimes revealing hypokalemia.

Aims: We reporting some observations informed by Multiple Myeloma complicated with a Fanconi syndrome.

Methods: From January 2000 till December 2010: 78 cases of Multiple Myeloma were brought together, whose circumstance of discovery 22 cases with renal failure, it's was a evolutes complications in 12 cases; and in 10 cases it's discovered at diagnosis. The renal achievement is dominated by Tubule disease in 11 cases, Randall syndrome 8 cases, and Nephrotic syndrome in 3 cases. The tubule disease of Fanconi is suspected at only 8 patients: in front of the presence of a renal glycosuria (without associated diabetes) and a frank proteinuria in the majority of the cases, with a hypophosphatemia and a fickle hypokalemia.

Results: The clinico-epidemiological and immuno-biological characters of these 8 patients are the following ones: - The median age is of 64 years (39-76), sex ratio=3. The osseous pains and the muscular cramps dominate the clinical presentation with constant diffuse demineralization in the radiology. - The patients were classified (according to the Salmon-Durie classification): IIIIB (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 5cases, terminal in 3cases. - We note more of hypocalcaemia while the hypercalcaemia is noted in a single case, the hypophosphatemia is found in half of the cases. The therapeutic attitude is double: - Symptomatic: alkaline hydration, correction of the metabolic disorders and sometimes the renal extra purge (indicated in 3cases). - Specific: chemotherapies VAD, a patient died by cardio-vascular complication. Under treatment the recovery of the renal function is obtained in 3 cases, to the rest 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 autopsique series. It is necessary to think to it in front of any renal achieve-vment 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 acidose 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 plateau dose was established as well tolerated and in the phase II part the study focused the efficacy and toxicity in the subgroup treated with maximum planned dose. The ASPIRE trial showed superior response rates and progression free survival for carfilzomib-lenalidomide-dexamethasone compared with lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients. The aims is explore the efficacy and tolerability of carfilzomib-lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients in real life.

Methods: All patients received carfilzomib 20/27 mg/m2 days 1,2,8,9,15 and 16; lenalidomide 25 mg days 1-21 and dexamethasone 20 mg days 1,2,8,9,15,16, 22 and 23, according to post approval access protocol. After 2, 4, 6, 8 cycles the responses, disease progression and toxicity were assessed using the International Myeloma Working Group Uniform Response Criteria and WHO score respectively.

Results: From January 2016 to February 2017 in hematology “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 12-92); median of prior therapy was 3 (range 1-6); 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 ≥PR, of these 3 VGPR. After 4 cycles, 5 (33%) and 1 (7%) have obtained at least a VGPR and CR respectively (Figure 1). Three patients were not evaluated for treatment discontinuation because of rapid progression disease and died during first cycle with a median of 5 prior lines therapy. Most grade 3-4 adverse events were haematological and well manageable, 10 (80%) trombocitopenia and 5 (35%) neutropenia grade 3-4. Dyspnea, fatigue and pyrexia were higher but were mostly grades 1 and 2. Only 2 patients developed respiratory failure and pneumonia while cardiac failure, ischemic heart disease and hypertension not were detected.

Table.1: Baseline patient characteristics.

<table>
<thead>
<tr>
<th>MEAN OF AGE, years (range)</th>
<th>MULTIPLE MYELOMA, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>62 (38-79)</td>
<td></td>
</tr>
<tr>
<td>REFRACORY</td>
<td></td>
</tr>
<tr>
<td>MULTIPLE MYELOMA SUBGROUP, n (%)</td>
<td></td>
</tr>
<tr>
<td></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, (n)</td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>3 (30)</td>
</tr>
<tr>
<td>III</td>
<td>12 (90)</td>
</tr>
<tr>
<td>II-III</td>
<td>7 (70)</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 (1-6)</td>
</tr>
<tr>
<td>Prior TRAPLANT, n (%)</td>
<td></td>
</tr>
<tr>
<td>Autologous</td>
<td>9 (60)</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Prior Therapy, n (%)</td>
<td></td>
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<tr>
<td>LENALIDOMIDE</td>
<td></td>
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<tr>
<td>Bortezomib</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Pomalidomide</td>
<td>15 (100)</td>
</tr>
<tr>
<td>8 (53)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: Carfilzomib-lenalidomide-dexamethasone is a powerful and efficay approach in relapsed/refractory Multiple Myeloma patients, which allows the achievement of deep responses from the first cycle of therapy. Non haematological adverse events of grade 3 or higher were reported in only 2 patients.
Aims: In this retrospective observational trial, it has been evaluated efficacy and tolerance of Carfilzomib, in combination with lenalidomide-dexamethasone (KRD) as salvage regimen in patients with relapsed and refractory MM (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 by the new treatment. 57% of them had under- and in particular one del13q14 1qgain, one del 13q14 and one t(11;14). 86% of patients had previously been treated with schedule containing bortezomib and IMIDs, and 33% had also received radiotherapy. 57% of them had undergone at least a single autSCT.

Results: Carfilzomib was well tolerated, with grade 2 anemia in 28% of patients, without necessity for blood transfusions; 5% grade 1 and 9.5% grade 3 neutropenia (no oespinalization 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, treated by common antibiotic drugs; grade 2 dyspnea in 5% of patients, treated by common antibiotic drugs; grade 2 Hypertension in 24% of patients; grade 3 arrhythmias in 5% of patients; grade 2 dyspnea in 5% of patients; grade 1 fatigue in 9.5% of patients. According to IMWG criteria, after a median follow-up of 3 months (r.1-13), ORR was 66.7% (16/24: 10 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 autSCT. Median time to response was 2 months (r:1-4), median OS from diagnosis was 47 months (9-170 range), median OS from start of Carfilzomib was 3 months (range 1-13).

Table 1.

PB2021

IMWG '14 DIAGNOSTIC CRITERIA TO INITIATE TREATMENT IN NEW DIAGNOSED MULTIPLE MYELOMA: REAL-WORLD STATISTICS

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Background: Diagnostic criteria for Symptomatic Multiple Myeloma (MM) Published in 2003 by the International Myeloma Working Group(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 Plasmaymoma) 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 mutlitoopic involvement. 7 of these NDMM were smoldering MM (sMM). All of them 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 criteria and the last one with BM Plasmatic Cell (BMPC) >60%, MRI image and FLC criteria. Although these data are quite different from those reported previously, accurate diagnosis in initial stages may increment the proportion of real-active MM. We don’t observe increments in incidence rate in these period vs pre´2014 (reported to 22nd EHA abstract). We observe that the early mortality is decreasing in the last 5 years (from 2013). The effect of early diagnostic may contribute to get these improvement of survival.

Summary/Conclusions: One of the hypotheses for introducing new criteria for initiating treatment was that the initiation of adequate 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 and WM were included. Four were classified as high-risk myeloma (Patients 1-4). Patient 1 (P1) had plasma cell leukemia and received Pom/dex plus bortezomib; Patient 2 (P2) presented complex karyotype and received Pom/dex after three previous regimens and an autologous transplantation; Patient 3 and Patient 4 (P3 and P4) had extramedullary plasmacytoma and received Pom/dex/local radiotherapy. The eight patients of this study had failed to bortezomib and lenalidomide-based therapy, and received Pom/dex until disease progression or unacceptable toxicity. Pom/dex was associated with ciclophosphamide in two patients, and with bortezomib in another two patients. The primary endpoint was progression-free survival (PFS).

Results: The median number of prior regimens was 2 (range, 1-4) and five of eight patients (62.5%) had previously received autologous transplantation. Median time from diagnosis to Pom/dex was 51.5 months (range, 28-155). Patients received a median of 6 cycles of Pom/dex (range, 2-16). In the whole series, the median follow-up was 60.5 months (IQR: 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% (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 somatically acquired in inpatient response. CEL-NOS patients negative for PDGFRαβ abnormalities (Jurko 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 test tailed test was used to compare the allele frequency between FIP1L1-PDGFRα 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 (0.096 versus 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 LEUKOCYTOSIS


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Background: Idiopathic leukocytosis and erythrocytosis are hematological disorders without specific causes. Frequent V617F mutations on the JAK2 gene have been reported in patients with polycythemia vera (PV), essential thrombocythemia, and primary myelofibrosis. We also found JAK2 V617F mutations in one of 11 patients with idiopathic erythrocytosis. Mutations of the CSF3R, SETBP1, 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. Accordingly we previously analyzed the JAK2, CSF3R, CALR, SETBP1, and ETNK1 genes in 10 patients with idiopathic leukocytosis (EHA20). To elucidate the relevance of genetic alterations, we extended the analysis with 17 genes known to be involved in hematological neoplasms in 16 patients with idiopathic leukocytosis.

Methods: Leukocytosis is defined as a total white blood cell count more than two standard deviations above the median or over 11,000/μL. Those patients who satisfied the following criteria were included in the study: leukocytosis (predominantly neutrophils); the absence of apparent causes of leukocytosis; and documentation of the leukocytosis over a prolonged period.
of time. The period of observation was 1 year or longer in most patients. Sixteen patients with idiopathic leukocytosis were analyzed in the study. Neutrophils or mononuclear cells were collected after obtaining written informed consent from the 16 patients. Neutrophils from peripheral blood were purified by dextran sedimentation followed by hypotonic lysis and centrifugation with Ficoll-Conray. Mononuclear cells were isolated from bone marrow by Ficoll-Conray gradient centrifugation. DNA was extracted using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA), Mutations within hot spots of the CSF3R, JAK2, CALR, SETBP1, ETNK1, CBL, TET2, ASXL1, EZH2, IDH1/IDH2, DNMT3A, U2AF1, and CEBPA genes were analyzed by direct sequencing in both directions using a 3730XL DNA Analyzer (Life technologies, Carlsbad, CA, USA) and/or allele specific polymerase chain reaction analysis. Total RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) were performed between the ET6V 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 leukocytosis. No mutations were found in the other genes in the 16 idiopathic leukocytosis patients. ET6V-ABL1 fusion gene was detected in one of the 10 patients. No BCR/ABL1 fusion gene was detected in the 8 patients. One idiopathic leukocytosis patient with JAK2 V617F mutation has developed PV. Another patient with sustained leukocytosis for 20 years showed cytogenetic abnormalities during observation and has developed Philadelphia chromosome negative CML (Ph-CML). ET6V-ABL1 fusion gene was detected in this patient. Another patient with normal karyotype progressed to blast crisis of Ph-CML characterized by cytogenetic abnormalities. Of the remaining 13 patients with idiopathic leukocytosis, one resolved the disease and twelve had a stable disease.

Summary/Conclusions: Idiopathic leukocytosis comprises heterogeneous conditions. JAK2 mutations and cytogenetic abnormalities are predictive of progression to hematological neoplasms.

PB2025
EVALUATION OF EXPRESSION OF MiRNAs ISOLATED MICRONEVESICLES OF PATIENTS WITH MYELOFIBROSIS ASSOCIATED WITH DISEASE L. Rodrigues1, J. Barros1, A. Nonino1, C. Mascarenhas1, 2
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Background: Myelofibrosis is a hematological disease inserted in the group of myeloproliferative neoplasias. It has as main characteristic fibrosis of the bone marrow, consequence of a variety of histological changes presented in the medullary microenvironment. The development of the disease is related to the clonal expansion of myeloid stem cells, abnormal expression of cytokines, hypercellularity of myeloid lines and also extramedullary hematopoiesis. The pathophysiology of myelofibrosis involves the activation of signal transduction pathways, which may occur due to genetic rearrangements and mutations that alter the structure of protein tyrosine kinases, making hematopoietic progenitor cells independent or hypersensitive to cytokines, generating anomalous cellular behavior. Myelofibrosis is a hematological disease inserted in the group of myeloproliferative neoplasias. It has as main characteristic fibrosis of the bone marrow, consequence of a variety of histological changes presented in the medullary microenvironment. The development of the disease is related to the clonal expansion of myeloid stem cells, abnormal expression of cytokines, hypercellularity of myeloid lines and also extramedullary hematopoiesis. The pathophysiology of myelofibrosis involves the activation of signal transduction pathways, which may occur due to genetic rearrangements and mutations that alter the structure of protein tyrosine kinases, making hematopoietic progenitor cells independent or hypersensitive to cytokines, generating anomalous cellular behavior.

Aims: Recent studies have shown that microRNAs produced by the cells of the organism may be associated with the cellular communication process due to their role in the regulation of translation and post-transcriptional regulation of gene expression. MicroRNAs present in the microvesicle content may col-
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 (MLL) and calreticulin (CALR), have been described in most patients with BCR-AbL negative MPNs. JAK2 mutations are present virtually all cases of Polycythemia Vera and 50-60% of prMF and Essential Thrombocythemia (ET). Recently, mutations in CALR gene were found in 60-80% 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: Bone marrow biopsy samples from 32 patients previously diagnosed as primary myelofibrosis with known JAK V617F mutation status were reviewed 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, Diaanova, 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 patients with 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 and MDR1 (MDR1) and calreticulin (CALR) were seen in 8 (47%) of patients with prMF 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 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

Aims: To evaluate mRNA expression of HIF-1α and HIF-2α in patients with JAK2 V617F positive MPN.

Methods: Real-time PCR was performed to detect mRNA expression levels of HIF-1α, HIF-2α, CALR, and MDR1 genes in whole blood samples from patients with JAK2 V617F positive MPN.

Results: Ct method in the software package of “R”. The threshold cycles (Ct) genes and housekeeping genes (TBP, GUS, ABL) determined using Ct method. The results was normalization with this reference genes. Mann-Whitney U test was used to evaluate significant difference between the groups, the degree of correlation (r) was assessed using Spearman test.

Results: We observed a lower mRNA expression MDR1 and CALR in whole blood samples of patients with MPN compared with a group of healthy volunteers (Figure 1). The expression level of mRNA HIF2α was not changed and for HIF1α it should be noted a tendency for statistical significance. It found no correlation between allelic burden and mRNA expression level. Index HIF 1α/2α more clearly showed a correlation with the fall of MDR1 and CALR mRNA levels (r=-0.64 in control and r=-0.7 in MPN group, p<0.05). CALR unlike MDR1 gene is not known among the target HIF regulation, but their unidirectional change indicates the possible metabolic links.

Figure 1.

Summary/Conclusions: We assume that the studied gene expression changes reflect the metabolic processes in the bone marrow progenitor cells. Probably JAK2 V617F mutation leads to more favorable microenvironment and reduced willingness to autophagy, causing the index shift HIF1α/2α. We found reduced of mRNA CALR expression in blood cells at MPN and this fact require further investigation.

PB2029

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 peripheral blood neutrophils for the diagnosis of either entity.

Methods: The 213 subjects were organized into 4 main groups; benign neutrophil leucocytosis 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: We observed a 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. Polycythemia 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, 5.85-25.7; 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 most cases. With a 20 p.d.u cutoff, the specificity of neoprophil CD177 MFI in Philadelphia-negative cMPDs patients’ diagnosis and determination 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.

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.
LYMPHOID LEUKEMIA.

with marginal zone lymphoma and a chronic myeloid leukemia with chronic

mutation of MPN and LPD: an essential thrombocythemia (ET) with myeloma, ET « Groupement Hospitalier Est », Lyon, France, of patients bearing an associ-

Aims:

We present 3 cases diagnosed in the Department of Hematology,

cause and all MPN are likely to present the onset of an associated LPD.

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

risk for MPN development. 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 risk for MPN development.

Background:

Chronic myeloproliferative diseases is a group of clonal Ph-neg-

ative hematological diseases, which include erythremia (polycythemia Vera, PI), chronic megakaryocytic leukemia (essential thrombocythemia, ET) and

subleukemic myelosclerosis (primary myelofibrosis, PMF), chronic idiopathic myelofib-

rosis). 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:

Detection of the frequency of occurrence of mutations in genes JAK2 and MPL and identifying the importance of the verification of these diseases. The study included 350 patients with chronic myeloproliferative dis-

ease. The frequency of occurrence of mutation V617F in the gene JAK2 exon 12

and MPL gene varies in different literature.

Methods:

The studied included 350 patients with chronic myeloproliferative dis-

eases — with polycythemia Vera 150 patients, with essential thrombocythemia 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 "AmpPrep RIBO-prep" (OOO Interlabservice, Russia). The concentration and purity of isolated DNA was determined by Nano Drop 2000 instrument (USA). Detection of gene mutation JAK2V617F and MPL gene was carried out by standard polymerase chain reaction on a thermal cycler 2720 "Applied Biosys-

tems" (USA), using a set of reagents "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 thrombocythemia in 42 patients of the 78 (54.2%), with chronic idiopathic myelofibrosis in 27 patients of 55 (49.1%). In 67 patients with no hematological profile, which were 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 MPL V615L mutation gene was detected in polycythemia Vera and chronic idiopathic myelofibrosis 2.2% (1 of 41) and 2% (1 of 52) of patients.

Summary/Conclusions: Thus established, our data confirm that mutations in the genes JAK2 and MPL are highly specific diagnostic markers in patients with Ph-negative chronic myeloproliferative diseases.

PB2030

DETECTION OF THE MUTATIONS IN GENES JAK2 AND MPL IN THE

ASSOCIATION OF MYELOPROLIFERATIVE NEOPLASM AND

LYMPHOPROLIFERATIVE DISORDER IN 3 PATIENTS

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tory, Groupement Hospitalier EST, 7Laboratoire, Centre Léon Bérard, Lyon, France

Background: Lymphoproliferative disorders (LPD) and myeloproliferative neo-

plasms (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 risk 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 thrombocythemia (ET) with myeloma, ET with marginal zone lymphoma and a chronic myeloid leukemia with chronic lymphoid leukemia.

Methods: Diagnosis have been made thanks to cytology of peripheral blood, bone marrow aspirate and biopsy and confirmed by cytogenetic and molecular biology techniques.

Results: Case number 1. A 68 year old woman known to have essential thrombocythemia as a MPN, with V617F mutation of the JAK2 protein kinase. After 19 years of treatment by Hydrea, she developed a splenomegaly, anaemia and slight lymphocytosis of 4.77 G/L. The blood smear, the bone marrow aspirate and biopsy examination revealed myelofibrosis evolution and an infiltration by 30% of a small sized clonal lymphoid population CD20+, CD5- Medullar karyotype was normal: 46, XX[10]. In conclusion the ET has evolved into myelofibrosis and is associated with a lymphoproliferative syndrome, possibly marginal zone lymphoma. No additional treatment has been implemented. Case number 2. A 64 year old woman know to have ET with V617F mutation of the JAK2 protein kinase treated by acetic salicylic acid. 5 years after, she presented with IgG kappa type monoclonal gammopathy up to 28 g/L, without any associated clinical manifestations or cytopenia. Medullar blood was diluted but showed slightly atypical plas-

mocytes remaining under 10%. Myeloma was diagnosed anyway and the patient received 5 cures of Velcade-Melphalan-Prednisone which resulted in complete remission. The MPN remains stable to this day. Case number 3. A 62 year old man with chronic lymphoid leukemia, treated by six cycles of R-FC. While in remission since 2 years, hemogram shows hyperleucocytosis (WBC: 18.3 G/L) with thrombocythemia (platelets: 1986 G/L) without anaemia (Hb: 13.7 g/dL). Blood smear examination reveals 3% of myeloma and basophilia (3.66 G/L). BCR-ABL transcript is positive in 43% and karyotype points out a 9;22 translocation. (46, XY, t (9 ;22) (q34 ;q11)[1]; nuc ish (BLX3, BCRX3,ABL.con BCRX2)[48/100].) Before starting Nilotinib, cytoreductive treatment by Hydrea was decided. Treatment is under way.

Summary/Conclusions: The three cases described highlight the diverse situa-
tions observed in cases of combined MPN/LPD pathologies. MPN with sec-
ondary onset of LPD are most frequently encountered, as was the case with patients 1 and 2. Cases of preexisting LPD and late onset MPN are rare (1), and cases of simultaneous discovery of both pathologies even more so (3). Several hypotheses have been formulated to explain the frequency of onset of these pathological associations: genomic instability due to JAK2 protein kinase activation, or due to BCR-ABL mutation, or exposure to cytotoxic chemotherapy or radiations (3).
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 main molecular marker in myeloproliferative neoplasms (MPN) and is harbored in about 50-60% of essential thrombocythemia (ET) and primary myelofibrosis (PMF). Recently, CALR mutation was described in ET and PMF. JAK2V617F and negative patients. There are two main variants of CALR mutation: type 1 (a 52-bp deletion) and type 2 (a 5-bp insertion).

Aims: To compare clinical and analytical data of ET and PMF patients with CALR type-1 vs CALR type-2 mutation.

Methods: We performed a single center study on 471 patients: 87 PMF and 384 ET. The JAK2V617F mutation was analyzed in DNA from peripheral blood leukocytes by PCR ARMS method. In all JAK2V617F negative patients detection of CALR mutation was performed by fragment length analysis and the results were confirmed by sequencing. Statistical data analysis was performed using statistica 12.5 software for Windows.

Results: From 384 ET patients 254 were JAK2V617F positive (66%), 80 were CALR positive (21%) and 51 were JAK2V617F and CALR negative (13%). From CALR positive patients: 36 (51%) had type-1, 34 (45%) type-2 mutation, and 10 (12%) type-3 mutation. From 87 PMF patients 56 were JAK2V617F positive (64%), 18 were CALR positive (21%) and 13 (15%) were JAK2V617F and CALR negative. From CALR positive groups: 13 (72%) had type-1 and 5 (28%) had type-2 mutation. Compared with ET carrying JAK2V617F mutation, patients ET CALR type-1 (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 (807 vs 800 G/L, p=0.007) but with no significant differences in frequency of thrombosis. In ET, CAL mutation was associated with increased odds of myelofibrotic transformation (odds ratio [OR]=2.61; 95% CI: 1.28 - 5.34; p=0.009) comparing with JAK2V617F positive patients. Patients ET CALR type-1 had higher leukocyte counts than ET CALR type-2 mutation (9.6 vs 7.3 G/L; p=0.008), but we did not find significant differences in hemoglobin, platelet counts, frequency of thrombosis or myelofibrotic transformation. Within PMF, no significant differences were observed. Moreover in PMF, there was no significant differences between the JAK2V617F, CALR type-1 and type-2 mutation status 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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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 (three-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+) – 133/30 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+), and 11/57 (19.3%) had bleeding events (hemorrhage+). Thrombotic complications in JAK2+ had 27.4% (50/182) pts, in TN – 30.7% (8/26) pts, in CALR1+ – 18.2% (2/11) pts and no cases of thrombosis were detected in CALR2+ and MPL+ subgroups (p>0.001). There were significant statistical differences in

Table 1. Number of line treatmentes required for disease control.

<table>
<thead>
<tr>
<th>Treatment lines</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76 (70.3)</td>
</tr>
<tr>
<td>2</td>
<td>23 (21.2)</td>
</tr>
<tr>
<td>3</td>
<td>7 (6.4)</td>
</tr>
<tr>
<td>4</td>
<td>1 (0.92)</td>
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<tr>
<td>5</td>
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</table>

Table 2. Drugs used in patients with ET.

<table>
<thead>
<tr>
<th>Treatment</th>
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</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>
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</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>Treatment</td>
<td></td>
</tr>
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</tr>
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</tr>
<tr>
<td>Danazol</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea + Anagrelide</td>
<td>2</td>
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</tbody>
</table>

Summary/Conclusions: This study highlights that, although ET has a very good prognosis, there is a significant percentage of patients that will need a change of treatment, either because of resistance or intolerance.

PB2033
ESSENTIAL THROMBOCYTHEMIA: STUDY OF TREATMENT LINES REQUIRED. EXPERIENCE OF A SINGLE CENTER
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Background: Essential thrombocythemia (ET) is a chronic myeloproliferative neoplasm that shows similar survival prognosis as general population, with a very low rate of transformation to myelofibrosis and acute leukemia. There are different treatments for these patients with optimal responses at first. For the first line, it is usually treated with hydroxyurea, although in young patients it is usually replaced by anagrelide / interferon. There are publications of hydroxyurea side effects, especially cutaneous, but there are not many studies about how many lines of treatment are needed to control the disease.

Aims: Study type and lines of treatment needed in patients with ET in a cohort of patients from January 1997 to January 2017.

Methods: We studied patients diagnosed of essential thrombocythemia in one area of the region of Murcia from January, 1997 to January, 2017. Those who started treatment and those who needed change were analyzed, either by resistance or by intolerance.

Results: In our area we have registered a total of 152 patients diagnosed with ET. Of these, 71% (108 patients) have required at least one treatment line. Table 1 shows the number of treatment lines required for the control of the disease. As it is shown in the Table, more than 20% of treated patients needed a second line and 6.5% required more than 2 lines. At last, Table 3 shows current treatment of ET patients.

Table 1. Number of line treatmentes required for disease control.

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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.
The DNA sequences extracted from the clinical samples were cloned into pGem with the additional stage of formation heteroduplexes was performed using the PyroMark Q24 (Qiagen, Germany). To verify the presence of mutations, exon 12-mutated PV also have a mutation JAK2V617 (<1%). JAK2 exon 12 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.

Summary/Conclusions: Leukocytosis >11x10⁹/l and thrombocytosis >1000x10⁹/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.

PB2035
DETECTION OF JAK2 EXON 12 MUTATIONS BY HETERODUPLEX ANALYSIS AND PYROSEQUENCING
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1Department of Medical Biology, Siberian Federal University, 2Krasnoyarsk branch of the Federal State budgetary Institution «Hematology Research Center» Department of Health, Krasnoyarsk, 3Federal Budget Institute of Science «Central Research Institute for Epidemiology», Moscow, 4Krasnoyarsk regional hospital, 5Municipal Budget Health Service Institution «City Clinical Hospital № 7», 6Krasnoyarsk Territory Department of Health Regional state budget health facility «Krasnoyarsk interdistrict clinic №1», 7Krasnoyarsk State Regional Bureau of Pathology, 8Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk, Russian Federation
Background: Somatic mutations in codons 533-547 of JAK2 exon 12 are highly specific to confirm the diagnosis of polycythemia vera (PV). We have previously proposed techniques for the detection and quantification of JAK2 exon 12 allele burden using a pyrosequencing method (Subbotina T et al, Haematologica, 2008). However, due to the high cost of sequencing, developing a two-stage algorithm for detecting mutations in JAK2 exon 12 using inexpensive screening approaches is of immediate practical necessity.
Aims: The aim of this study was to demonstrate the feasibility of using heteroduplex analysis with a secondary confirmatory test by electrophoresis on non-denaturing PAGE as the preliminary screening test for detection of JAK2 exon 12 mutations.
Methods: 274 patients with PV or unclear erythrocytosis and with a low JAK2V617F allele burden or unmutated JAK2V617 (51 women, mean age 52.2±15.7 years and 223 men, mean age 43.6±15.6 years) were included in this study. The informed consents from these patients were obtained. The PCR with the additional stage of formation heteroduplexes was performed using the Real-time PCR kit (Syntol, Russia) and CFX 96 Real Time System (BioRad, USA). PCR products were analyzed by electrophoresis in 8% PAGE. The presence of the mutations was identified and confirmed by pyrosequencing method with PyroMark Q24 (Qiagen, Germany). To verify the presence of mutations, the DNA sequences extracted from the clinical samples were cloned into pGem-T vector using standard protocol («Promega», USA), and obtained clones were sequenced using reagents and equipment of the «Applied Biosystems» (USA). JAK2 exon 12 varianceMUT was calculated as a measure of relative changes in allele burden between the baseline and follow-up sample (Theocharides A et al, Haematologica, 2008).
Results: We detected JAK2 exon 12 mutation in five out 274 patients. The results of electrophoresis on non-denaturing PAGE are reported in Figure 1. The type of №1-5 patient mutations was determined by pyrosequencing: N542-E543del (c.1624_1629delAATGAA); I540-E543delinsKK (c.1619_1627TCA-gAAATGK (c.1622_1627delGAAATG) and p.H538_K539L (c.1612_1616CACAAT-TT). These mutations have already been described. Main characteristics of 5 patients with JAK2 exon 12-mutated PV are reported in Table 1. The PV diagnosis of №1, 2, 3 and 5 patients was confirmed by bone marrow trephine biopsies histological examination. All five patients with JAK2 exon 12-mutated PV have an increased number of red blood cells, along with an accompanying increase in the concentration of hemoglobin and hematocrit level in the peripheral blood. Some of them had increase number of leukocytes and platelets in the disease dynamics. №1-4 patients was treated phlebotomy only and did not received any cytoreductive treatment to date. Patient №5 receives hydroxyurea (HU). Importantly, two out five patients with JAK2 exon 12-mutated PV also have a mutation JAK2V617 (<1%). JAK2 exon 12 allele burden in sample from №1 patient is significantly increased in the disease dynamics.

Summary/Conclusions: The proposed variant of the heteroduplex analysis with separation of the PCR product by electrophoresis on non-denaturing PAGE can be recommended for use as the preliminary screening test which is carried out before the confirming pyrosequencing. The two-stage approach allows to optimize the algorithm of the JAK2 exon 12 mutation detection and to improve the efficiency of testing for patients suspected of having PV in whom a JAK2V617F mutation is not detected or detected in a low allele burden. In five out 274 patients we detected JAK2 exon 12 mutation and confirmed the diagnosis of PV.

PB2036
INTRODUCTION OF AN NGS GENE PANEL INTO CLINICAL SERVICE FOR MYELOPROLIFERATIVE NEOPLASMS
A. Skowronska1,*, J. Bryon1, S. Clokie1, Y. Wallis1, J. Mason1, K. Reay1, M. Griffiths1
1West Midlands Regional Genetics Laboratory, Birmingham Women’s and Children’s NHS Foundation Trust, Birmingham, United Kingdom
Background: In the West Midlands region of the UK, all patients with a suspected myeloproliferative neoplasm (MPN) have access to quantitative analysis...
of JAK2 V617F by droplet digital PCR as standard of care. The British Committee for Standards in Haematology recommends that suspected MPN cases have investigation of JAK2 exon 12, CALR and MPL genes if JAK2 V617F is negative.

Aims: The aim of the project was to improve the MPN service by substituting sequential analysis of individual target regions within the JAK2, CALR and MPL genes with a single assay, and to increase the number of genes available for analysis.

Methods: A commercial next generation sequencing (NGS) gene panel (Oxford Gene Technology, SureSeq Myeloid Panel), coupled with the Illumina MiSeq platform was validated and implemented. The gene panel utilises hybridisation based enrichment technology and consists of 26 MPN-related genes. During the validation stage the following were enriched and analysed: 29 positive control samples with 30 known pathogenic variants, 30 negative control samples without known pathogenic variants in the JAK2, CALR and MPL genes, and 24 MPN samples of unknown mutational status. Thus so far over 200 clinical samples have been analysed and reported since the service was introduced in October 2016.

Results: The panel has successfully identified: a large range of known pathogenic variants at high sensitivity (JAK2 V617F variant allele frequency 1%, CALR Type 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 prevented the hybridization of the probe binding site of the JAK2 V617F ddPCR assay which had led to a false negative result by ddPCR. The validation procedure also explored coverage and limits of sensitivity, potential chemistry specific artefacts and identified common polymorphisms for all 25 genes.

Summary/Conclusions: The panel has replaced the current sequential analysis of CALR, MPL and JAK2 exon 12 in JAK2 V617F-negative patients and reduced turn-around-times with increased accuracy and sensitivity compared to Sanger sequencing and fragment analysis. Our current clinical service operates on a two tier system whereby clinicians can request analysis of the full 25 gene panel or a 4 gene subset (JAK2, CALR, MPL, CBL as an in silico analysis).

PB2037
IN JAK2V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS, BLEEDING RISK CORRELATES WITH ALLELE BURDEN
I. Bertozzi1, E. Cosi1, C. Santarossa1, G. Bognoli1, F. Fabris1, M.L. Randi1,1Dep. Internal Medicine - DIMED, University of Padova, Padova, Italy

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 thrombocytemia (ET) or primary myelofibrosis (PMF). JAK2V617F-positive patients display different clinical and molecular features from JAK2-WT patients, 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 hemorrhage (petechiae, epistaxis, menorrhagia and gingival hemorrhage). The impact of different allele burden on bleeding risk is uncertain.

Aims: Aim of our study is to explore whether there is an association between JAK2V617F allele burden and hemorrhagic complications in a large cohort of MPN diagnosed and followed in a single center.

Methods: We selected 253 MPN (121 ET: 47.8%, 124 PV:49% and 8 PMF:3.2%) carrying JAK2V617F mutation. The median follow-up of patients was 8.8 years (0.1 – 37.3 y). Complete medical history and anti-thrombotic drugs use were recorded. Hemorrhagic complications were classified as “major” or “minor” in agreement with ISTH criteria. The patients were categorized into four groups according to the amount of JAK2 mutant allele, (1st quartile 1-25%, 2nd quartile 26-50%, 3rd quartile 51-75% and 4th quartile 76-100%). Nominal variables were compared with X² test or Fisher’s exact where indicated. Survival has been evaluated only for groups with different prevalence of events during follow-up and were calculated with the Kaplan Meier method and compared with the log rank test.

Results: Three patients (1.2%) bled at diagnosis (1 major and 2 minor hemorrhages) while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages rates higher in 4th quartile compared both to 2nd (p=0.003) and to 1st (p<0.001) quartiles. Hemorrhages-free survival was higher in 1st quartile compared both to 2nd (p=0.004) and to 1st (p<0.001). The incidence rate of hemorrhages are respectively 0.7/100 patient-yrs for 1st quartile, 0.65/100 patient-yrs for 2nd quartile, 1.26/100 patient-yrs for 3rd quartile and 3.23/100 patient-yrs for 4th quartile with a IRR of 5 and of 4.6 for the 4th quartile respectively versus 2nd and 1st one. No statistically significant different has been found 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
M. Napolitano1, S. Siragusa1, M. Santoro1, M.R. Lanza Cariccio1, M. Bono2, F. Di Piazza2, A. Russo2, V. Accurso1
1UO Ematologia, 2Laboratorio di genetica e oncologia molecolare, 3UO Oncologia, University Of Palermo, Palermo, Italy

Background: The JAK2V617F allele burden (JAK-AB) plays a central role in chronic myeloproliferative neoplasms (cMPNs); its presence has also been advocated in the differential diagnosis of cMPNs and as independent risk factor for venous thromboembolic complications. New treatment with Ruxolitinib may decrease JAK-AB but at the present, it is not clear the clinical advantage of this treatment.

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, hemorrhagic and myeloid related events.

Methods: Peripheral blood samples from patients with Ph-negative cMPNs were collected, DNA from leukocytes 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 JAK-AB with demographic and clinical data.

Results: One hundred and twelve patients with Philadelphia negative cMPNs were investigated: 50 females with a median age at diagnosis of 69 years (age range: 18-95 years), 60 males with a median age of 68 years (age range: 18-82 years). Thirty-four patients had Essential Thrombocytosis (ET), forty-three patients 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 IPSET and DIPSS scores. In patients with PV (n=52), a significant correlation was observed 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 (PMFII and IV). JAK-AB had a positive correlation with splenomegaly in PMF.

Summary/Conclusions: Our report cannot confirm any correlation between allele burden and thrombotic risk, according to currently adopted scoring systems. A previous history of thrombosis is however associated with the highest AB in all cases.

PB2039
COMPARISON OF CLINICAL AND LABORATORY DATA, INCLUDING JAK-2 46/1 HAPLOTYPE, BETWEEN PATIENTS WITH IDIOPATHIC ERYTHROCYTOYSIS AND POLYCYTHEMIA VERA
M. Napolitano1, S. Siragusa1, M. Santoro1, F. Di Piazza2, M. Bono2, S. Mancuso1, A. Russo3, V. Accurso1
1UO Ematologia, 2Laboratorio di genetica e oncologia molecolare, 3UO Oncologia, University Of Palermo, Palermo, Italy

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 polycythemia 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 IE 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.
Summary/Conclusions: In the current study, we highlighted peculiar clinical and laboratory findings of IE, in comparison with Polycythemia Vera. As shown by available studies, Hb and HCT level do not easily discriminate between the two categories of patients while gene panels may be useful to improve diagnostic accuracy of IE. We have here first observed the presence of Jak-2 46/1 haplotype in approximately half patients with IE, even in absence of Jak-2 mutations; the homozygous status was statistically different among PV and IE patients. The role of such association deserves further specific studies.

PB2040
LABORATORY RESPONSIVENESS OF LOW-DOSE ASPIRIN IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA
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Background: The essential thrombocytopenia (ET) is a myeloid neoplasm characterized by platelet hyperreactivity and thrombosis. The daily low-dose aspirin (ASA) is a cornerstone in the prevention of the thrombotic events. In the ET an accelerated platelet turnover translates in a renewal of the drug target shortening the duration of cyclooxygenase (COX-1) inhibition and may dictate new dosing strategies particularly in ASA "low-responders" patients.

Aims: Therefore, we evaluated platelet count, β-thromboglobulin (β-TG) and platelet factor 4 (PF4), as markers of platelet activation, the platelet function activity (PFA), as indicator of ASA platelet sensitivity.

Methods: We enrolled 60 patients (20 men, 40 women; mean age 51 years range 32-70) with ET according to WHO criteria. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Of the 60 patients, 45 were on angiolide hydrochloride (daily dose 1.5 mg) (10 men, 35 women), 15 were on hydroxyurea (daily dose 2 mg) (10 men 5 women). None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelets were measured by automated analyzer. β-TG and PF4 were determined by ELISA. ASA platelet sensitivity was measured by Platelet Function Analyzer (PFA-100).

Results: The mean platelet count was 455±200x109/L. All patients had normal β-TG and PF4 (12±5 IU/ml and 4±1 IU/ml) and prolonged C/EPI closure time (T, unit: s, n.v. 84-160 s) (249±40 s).

Summary/Conclusions: These findings suggest that in ET patients the daily low-dose ASA represents an optimal dosing strategy and that PFA test may be an useful tool to distinguish between the ASA "normal-responder" and "low-responder" ET patient.

PB2041
CLINICAL AND EXPERIMENTAL CHARACTERISTICS OF MYELOID/LYMPHOID NEOPLASMS DISPLAYING PDGFRα OR PDGFRβ REARRANGEMENT
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Background: According to the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia, the cases with rearrangement of tyrosine kinase (TK) genes PDGFRα, PDGFRβ are classified in Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRα, PDGFRβ, or FGFR1, or with PCM1-JAK2. It is a rare event that patients presented remarkable hematological and clinical features. In the past decade, the dose of TKI to cases with PDGFRα and B abnormal was inconclusive.

Aims: The goal of the study was to assess the clinical and experimental characteristics and observe the response of Imatinib(IM) therapy of Myeloid/lymphoid neoplasms with PDGFRα or B abnormal.

Methods: Cytogenetic examination of bone marrow cells obtained from patients was performed by 24h culture method. R banding technique was used for karyotype analysis. PDGFRα and B gene rearrangement were detected by FISH using triple-color of 4q12 and dual color break-apart PDGFRβ probes.

The fusion genes of rearrangements of PDGFRα 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 PDGFRα rearrangement, the other 8 were PDGFRβ 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 PDGFRβ rearrangement had a primary abnormality with t(5;12)(q33;p13) and the other one had a secondary abnormality of AML-M2. PDGFRα and B genes rearrangement detected by FISH and multiple-RT-PCR were positive. The immunophenotypical analysis showed myeloid or lymphoid. The FeRenda achieve rapid and durable remissions. IM.

Summary/Conclusions: In summary, patients with significantly anemia and eosinophilia should be screened for the presence of PDGFRα and B rearrangements. The dual-color FISH is a simple approach and should be added into the diagnostic work-up because these patients respond to imatinib therapy, and sustained responses have been observed. The OS of PDGFRα and B abnormal was similar with a previous report in a western population and another Chinese hematology center.
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 with associated neoplasms (SMAN) (n=3), mast cell leukemia (MCL) (n=4) and mast cell activation syndrome (MCAS) (n=1).

**Results:** At diagnosis, age of patients with advanced disease was higher than ISM and SSM group (p=0.001). Most frequent symptom of disease was skin lesion (urticaria pigmentosa) (%64). Skin lesions were significantly higher in patients with ISM and SSM than with advanced disease (p<0.009). But B symptoms were significantly higher in advanced disease variant (p=0.013). Anemia, trombocytopenia, elevation of ALP and GPT, hypoalbuminemia were significantly higher in advanced disease than in ISM and in SSM. Osteopenia was higher in patients with ISM and SSM than with advanced disease, %56 and %18 respectively. KITD816V mutation was detectable in peripheral blood in 33 of 40 mastocytosis patients (%82) with a median Ct value 36±4. Median Ct value was significantly lower in advanced SM (Ct: 32±5) than in ISM 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**

**COMPARISONS OF PATIENT MANAGEMENT IN MYELOPROLIFERATIVE NEOPLASM PATIENTS IN THE UK VS REST OF WORLD: ANALYSIS FROM THE INTERNATIONAL LANDMARK SURVEY**

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**Background:** Patient (Pts) with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocytosis (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated the patient-reported impact of MPNs in pts across 6 countries and identified current treatment strategies in these pts. Pts to manage their MPN 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 administered 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 treatment patterns and patient physician communication.

**Results:** A total of 699 pts (UK, n=286; ROSW, n=413) and 219 physicians (UK, n=31; ROSW, n=188) completed the survey. UK physicians were more likely to report to 10% more patients than ROSW. UK physicians reported that their patients were more likely to ‘often’ disagree with their primary physician on treatment decisions than ROSW. This difference was greater in PV and ET than MF. For MF the most commonly received treatments were ruxolitinib (UK 55%, ROSW 50%), aspirin (UK 53%, ROSW 37%), hydroxyurea (HUK) (UK 31%, ROSW 28%) and transfusion (UK 27%, ROSW 23%), for PV they were aspirin (UK 83%, ROSW 58%), phlebotomy (UK 76%, ROSW 67%) and HU (UK 63%, ROSW 36%) and for ET they were aspirin (UK 94%, ROSW 52%), HU (UK 62%, 30% ROSW) and anagrelide (UK 14%, ROSW 18%). Physician reported data on treatments prescribed demonstrated a similar pattern as a greater proportion of UK physicians reported using treatments than ROSW. UK physicians reported that their patients were significantly more inclined to being involved in treatment decisions (mean: UK, 6.25; ROSW, 7.01). UK patients supported this change as more ‘agreed strongly’ with the statement ‘I involve my MPN patients in treatment decisions’ (UK, 39%; ROSW 28%).

**Summary/Conclusions:** In comparison with ROSW UK physicians were more likely to prescribe drug treatments for ET/PV. Interestingly, UK patients reported to be more involved in treatment decisions, and this was reflected in the physician’s perspective to involve their patient in treatment decisions more. UK patients were also more likely to disagree with their physician on primary treat-
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 likely 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 of 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 (ASCT) reported that allele burden of 2.9% after 20 days of transplant and a negative result after 60 days of transplant vs 13% before ASCT. CALR mutation is found in ET and PMF cases that are mutually exclusive with JAK2V617F and MPL exon 10 mutations in ET whereas 2 cases with PMF found to be positive for JAK2V617F and CALR mutations. We found 40 cases with a 52-bp deletion, 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 65 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 11 515L/K 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.

Summary/Conclusions: We analyzed JAK2/MPL/CALR genes as molecular marker’s for MPN’s, allows the diagnosis of 95% of patients with MPN. As a novel mutation, CALR testing also has a prognostic significance and it was not mutually exclusive with JAK2V617F mutation. Measurement of JAK2 V617F allele burden early after transplantation is an important predictive parameter in monitoring patients following this treatment. The knowledge of driver mutations can provide valuable information for diagnosis and prognosis, which ultimately can be highly useful for clinical decision making for the management of patients with MPN.

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 PMF.

Methods: A multicenter retrospective study was carried out. Samples of peripheral venous blood was obtained from 149 patients with ET (n=76) and PMF (n=73). Patients that were negative for JAK2V617F and MPL515L/K mutations were studied for CALR mutations presence as described in original paper (TKlampf, 2013). CALR Mutations were detected in 34 patients with ET (10 - type I, 24 - type II) and 25 patients with PMF (13 - type I, 12 - type II). Statistical data processing was carried out in the program STATISTICA for Windows 6.0.

Results: The frequency of mutations CALR was comparable in patients with ET and PMF (44.7% and 35.6%). Mutations of type II is 2 times more common in ET than with the TFM: 17.1% vs 9.6% (p=0.178), Mutations of type 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 of CALR (p=0.014). Spleen size in ET patients on the time of the diagnosis date was slightly different: in type I - 106.5mm, type II - 119.6mm (p=0.076). The type of mutation in our study had no effect on the stratification according to the IPSS. 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 more pronounced 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 molecular 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 mutations 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.

PB2049
Abstract withdrawn.

PB2049
THE UNIQUE CASE OF GELERINE CEBPA MUTATION IN PATIENT WITH FIP1L1/PDGFRα 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/PDGFRα (F/P) fusion gene results from a cryptic interstitial deletion at 4q12 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 lymphoma, mast-cell (T-cell) leukemia (TML), aggressive systemic mastocytosis (ASM), (MLNe) with eosinophilia and Philadelphia chromosome (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. CCAT/enhancer binding protein alpha (CEBPA) gene functions as key regulator of granulocytic differentiation and CEBPA mutations have been detected in association with 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 (20x10^9/L), with marked eosinophilia (4,0x10^9/L). A bone marrow aspirate showed 2% blasts, 21% eosinophils. Histological examination of an excised lymph node biopsy showed 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 clonal 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 transplants from his brother. Routine testing of chimerism at 2 months after HSCT revealed the recipient DNA less than 5% and positive probe for F/P and CEPBA. We hypothesized the germinal origin of CEPBA mutation.

Results: The same N-terminal (TAD2) CEPBA mutation was found in the patient’s skin, male node and bone marrow, and in the patient’s brother bone marrow samples. Unfortunately, no materials from parents was available for analysis at that time.

Summary/Conclusions: Germline CEPBA 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 patients with PFDGFR-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 NEOPASMS

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Background: Patients with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocytopenia (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 World (ROW).

Methods: A cross-sectional survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK. The internet-based survey was administered separately to MPN patients and treating physicians (the two samples were not linked). Observed differences between the UK and ROW are described in terms of symptom burden.

Results: A total of 699 pts (UK, n=286; ROW, n=413) and 219 physicians from UK and ROW, n=190) completed the survey. UK patients reported more symptoms than those in ROW (9.02 vs. 5.95 respectively). A higher proportion of UK patients reported experiencing symptoms compared with ROW (e.g. fatigue and tiredness UK – 87% MF and PV, 86% ET; ROW – 64% MF, 39% PV, 45% ET). This pattern was observed for 28 of the 31 symptoms recorded. A similar difference was seen when physicians were asked about frequency of patient-reported symptoms (e.g. fatigue and tiredness UK – 90% MF, 67% PV, 70%; ROW – 71% MF, 55% PV, 48% ET). Patients rated symptom severity from 1 (not severe at all) to 10 (worst possible). The UK was higher than ROW for the three most common symptoms: fatigue and tiredness (mean: UK 6.73, ROW 5.38), pain (mean: UK 6.06, ROW 5.38) and number of symptoms (mean: UK 6.01, ROW 5.67). 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 (UK > 9% compared with 8% of ROW), and less likely to report never responding after completing the informed consent (n=2). Outcomes will be complete by May 8, 2017 with results to be published at EHA.

Summary/Conclusions: Data presented here will inform next steps for a RCT investigating the effectiveness of online yoga for symptom management in MPN patients.

PB2051

COMPARISONS OF SYMPTOM BURDEN IN MYELOPROLIFERATIVE NEOPASMS IN THE UK VS REST OF WORLD: ANALYSIS FROM THE INTERNATIONAL LANDMARK SURVEY


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Background: Patients with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocytopenia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated patient-reported impact of MPNs across 6 countries.

Methods: A cross-sectional survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK. The internet-based survey was administered separately to MPN patients and treating physicians (the two samples were not linked). Observed differences between the UK and ROW are described in terms of symptom burden.

Results: A total of 699 pts (UK, n=286; ROW, n=413) and 219 physicians from UK and ROW, n=190) completed the survey. UK patients reported more symptoms than those in ROW (9.02 vs. 5.95 respectively). A higher proportion of UK patients reported experiencing symptoms compared with ROW (e.g. fatigue and tiredness UK – 87% MF and PV, 86% ET; ROW – 64% MF, 39% PV, 45% ET). This pattern was observed for 28 of the 31 symptoms recorded. A similar difference was seen when physicians were asked about frequency of patient-reported symptoms (e.g. fatigue and tiredness UK – 90% MF, 67% PV, 70%; ROW – 71% MF, 55% PV, 48% ET). Patients rated symptom severity from 1 (not severe at all) to 10 (worst possible). The UK was higher than ROW for the three most common symptoms: fatigue and tiredness (mean: UK 6.73, ROW 5.38), pain (mean: UK 6.06, ROW 5.38) and number of symptoms (mean: UK 6.01, ROW 5.67). 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 (UK > 9% compared with 8% of ROW), and less likely to report never responding after completing the informed consent (n=2). Outcomes will be complete by May 8, 2017 with results to be published at EHA.

Summary/Conclusions: Data presented here will inform next steps for a RCT investigating the effectiveness of online yoga for symptom management in MPN patients.
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; they are also more likely to think they don’t have enough time to discuss all symptoms. This is likely to be impacting on the ability of patients and physicians to communicate fully on symptoms and to agree on the best disease management plan.

PB2052

MPN10 SCORE AND SURVIVAL OF MOLECULARLY ANNOTATED MYELOPROLIFERATIVE NEOPLASMS PATIENTS; A FIRST REPORT ON AN EGYPTIAN COHORT

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Background: The vast majority of myeloproliferative neoplasms (MPNs) patients are characterized by a molecular genetic background and by variable symptoms reflecting disease burden that may correlate with prognosis.

Aims: To study the impact of driver gene mutations: Janus kinase 2 (JAK2), calreticulin (CALR) and myeloproliferative leukemia virus oncogene (MPL) on disease burden and correlating mutational status with symptom severity calculated by MPN10 score, degree of bone marrow (BM) fibrosis, clinical characteristics and survival in MPNs patients.

Methods: MPNs Symptoms Assessment Form Total Symptom Score (MPN-SAF TSS) was assessed as mean/median of 10 items: fatigue, concentration, early satiety, inactivity, night sweats, itching, bone pain, abdominal discomfort, weight loss and fever. JAK2V617F and exon12 mutations were performed by allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) while CALRexon9 insertion/deletion and MPL W515exon10 mutations were assessed by high-resolution melting (HRM).

Results: 93 MPNs patients (48 males and 45 females): 18 polycythemia vera (PV), 41 essential thrombocythemia (ET), 24 primary myelofibrosis (PMF), 10 Post-EVT/PV-malignant fibrosis (post-EVT/PV-MF) were included. Median age at diagnosis was 55 years (17-75) and was lower in ET than PV and PMF patients; 44 (19-75) vs 56 (34-70) years and 56 (20-75) years, respectively (p<0.001). JAK2 mutation was positive in 3/93 (3%); 2 (5%) ET patients, 1 (4%) PMF patients, 4 (17%) PMF patients, zero (0%) Post-ET/PV-MF patients (p<0.050). MPL mutation was positive in 14/93 (15%); 10 (24%) ET patients, 4 (17%) PMF patients, zero (0%) Post-EVT/PV-MF patients (p=0.011). CALR mutation was positive in 14/93 (15%); 10 (24%) ET patients, 4 (17%) PMF patients, zero (0%) Post-EVT/PV-MF patients (p=0.050). MPL mutation was positive in 3/93 (3%); 2 (5%) ET patients, 1 (4%) PMF patients, zero (0%) Post-EVT/PV-MF patients. 23/93 (25%) patients were triple negative; 15 ET, 2 PV, 4 PMF and 2 post ET-MF. Median MPN10 score was 21 (4-45) in ET versus 37.5 (25-56) in PV, 54 (15-80) in PMF and 59 (45-75) in Post-EVT/PV-MF (p<0.001). From the patients with BM fibrosis, 6 (15%) were triple negative vs 33 (85%) mutant patients (p=0.007). Among 52 patients with splenomegaly, 7 (13.5%) patients were triple negative vs 45 (87%) patients with a positive mutational status (p<0.001). Median MPN10 score was 48 (5-76) in JAK2 positive patients vs 25 (4-80) in JAK2 negative (p<0.001) and was 22.5 (4-65) in CALR mutants vs 35 (5-80) in CALR negative (p<0.050). Median MPN10 score was 21 (10-48) in triple negative patients vs 40 (4-80) in MPNs JAK2/CALR/MPL mutants (p<0.001).After a median follow-up period of 36 months (6.102), progression free survival (PFS) and overall survival (OS) of the whole cohort was 85% and 95%, respectively. PFS of JAK2 positive vs negative patients was 62% vs 0% (p<0.001), PFS of CALR positive vs negative patients was 100% vs 78% (p=0.067). PFS of triple negative vs mutant patients was 100% vs 75% (p=0.004). OS of JAK2 positive vs negative patients was 85% vs 100% (p=0.011). PFS of CALR positive vs negative patients was 100% vs 92% (p=0.197). OS of triple negative vs mutant patients was 100% vs 90% (p=0.015) (Figure 1).

Summary/Conclusions: MPN10 score is directly affected by JAK2 and CALR positivity and can be used as a major predictor of survival in 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.

PB2053

FINAL RESULTS FROM PEN-PV STUDY, A SINGLE-ARM PHASE 3 TRIAL ASSESSING THE EASE OF SELF-ADMINISTRATING ROPEGINTERFERON ALFA-2B USING A PRE-FILLED PEN IN POLYCYTHEMIA VERA PATIENTS


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Summary/Conclusions: 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. Ropeginterferon alfa-2b (AOP2014) is a novel long-acting monopeptide 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.

Aims: Open-label, single arm, multicenter phase III trial assessing the self-administration of AOP2014 using a pre-filled, dose-adjustable pen (NCT. 2014: 001356-31).

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 (4 supervised self-administrations at site, followed by 3 self-administrations in the home-setting, and a 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 (15/36 (41.7%)) entering the study received a baseline dose of 500 µg AOP2014. 72.2% (26/36) used 2 pens (dose >250 µg) and 27.8% of patients (10/36) used one pen (dose up to 250 µg) to administer the appropriate dose. At the first supervised visit, 80.6% (29/36) of patients had achieved full success, defined as no technical problems with the pen experienced by the patient during the injection, and no early withdrawal of the pen (before injection was complete), both observed by the investigator. At the second supervised visit full the success rate was 91.7% (33/36). The majority of observations resolved after the second supervised visit. Only 5 patients (13.9%) needed one additional supervised visit prior using the pen correctly in a home-setting. All patients had achieved full success at the third supervised visit at home (final study visit).

The patients responded favourably to the use of the pre-filled pen for the administration of AOP2014 and the accompanying instructions. Based on the Investigator’s assessment, no patients exhibited any visible pain or physical discomfort, appeared to be dissatisfied when using the pen or exhibited any frustrations. Most patients (32/36 patients) rated the instructions for the AOP2014 pen (i.e. scope and structure of the leaflet, clarity and comprehensibility of the text, clarity of the images and design of the leaflet), and the AOP2014 pen itself (i.e. setting the dose, user-friendliness, injection procedure) as “satisfactory” or “good”. That the hematological parameters and spleen size remained stable throughout the study, and the rate of responders (haematological response with and without spleen size) was maintained during the entire study, suggesting that the use of the pen device did not affect drug activity. Of the 47 adverse events (AE) reported during the study, 19 were related. Most AE were classified as moderate in intensity. One serious AE (treatment related, unrelated), one pen-relaeved AE (mild nervousness reported prior first administration in the home setting), and one Grade 3 TEAE (pain in extremity, related) were recorded, but none led to a dose reduction.

Figure 1.
Summary/Conclusions: The AOP2014 pen was well accepted and no major difficulties were reported. The study drug performed as expected and there were no safety concerns arising from the administration of AOP2014 using the pen device. The AOP2014 pen allows for individual dosing and a patient-convenient mode of self-administration of rGDF-21 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 BIOSPY

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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 myeloproliferative neoplasms (MPNs). Consistent with known literature, the molecular characterisation have implications in the phenotype 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 where 55 (72%) patients JAK2, 12 (15.5%) patients CALR, one patient MPL and 9 (11.8%) patients triple-negative (TN). The main clinical and laboratory features of the patients are 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 megacaryocytes according with the mutational status and there were more frequently 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>No.</th>
<th>Genotypic</th>
<th>Subgroup</th>
<th>Initial JAK2 V617F allele (%)</th>
<th>Follow-up JAK2 V617F allele (%)</th>
<th>Difference</th>
<th>Initial JAK2 V617F allele (%)</th>
<th>Follow-up JAK2 V617F allele (%)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>PV</td>
<td>15.20</td>
<td>53.64</td>
<td>-37.07</td>
<td>15.76</td>
<td>53.64</td>
<td>VITAMIN E</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>PV</td>
<td>46.11</td>
<td>76.28</td>
<td>-30.17</td>
<td>16.00</td>
<td>76.28</td>
<td>VITAMIN E</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>PV</td>
<td>61.56</td>
<td>41.55</td>
<td>-20.11</td>
<td>11.74</td>
<td>41.55</td>
<td>FLUDARABINE</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>PV</td>
<td>40.00</td>
<td>46.95</td>
<td>-6.95</td>
<td>10.00</td>
<td>46.95</td>
<td>Fludarabine, cyclophosphamide</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>PV</td>
<td>11.50</td>
<td>4.94</td>
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<td>4.94</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>ET</td>
<td>38.10</td>
<td>30.33</td>
<td>-8.27</td>
<td>4.38</td>
<td>30.33</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>ET</td>
<td>21.29</td>
<td>16.92</td>
<td>-5.27</td>
<td>0.00</td>
<td>16.92</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>ET</td>
<td>0.17</td>
<td>0.00</td>
<td>-0.17</td>
<td>0.00</td>
<td>0.00</td>
<td>Hydroxyurea</td>
</tr>
</tbody>
</table>

* Data from the first follow-up sample.  † Data from the next follow-up sample in the same patient.

Figure 1.
Summary/Conclusions: Quantitative analysis of JAK2 mutation using ddPCR was highly correlated with pyrosequencing and might reflect clinical treatment response.

PB2056

CLINICAL IMPACT OF JAK2 AND CARLETICULIN GENE MUTATIONS ON PATIENTS WITH ESSENTIAL THROMBOCYTHYMIA

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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 involving codon 515. Recently, mutations at the exon 9 of calreticulin (CALR) gene have been identified in approximately 50% of patients with ET, unmutated for JAK2 and MPL.

Aims: Primary aim of the current study was to analyze the prevalence of JAK2, MPL and CALR gene mutations in patients with ET; secondary aim was to evaluate the impact of gene mutations on clinical features of ET at diagnosis.

Methods: A cohort of consecutive patients with a diagnosis of ET followed between January 2013 and June 2016 were considered. JAK2 (V617F) gene mutation was detected by PCR testing; MPL and CALR mutations were analyzed by direct sequencing methods. Thrombotic risk score was calculated according to European Leukemia Net recommendations. Data were statistically analyzed.

Results: Overall, 148 patients were included: 107 (72.30%) had JAK2 (V617F) gene mutation (JAK2+), 12 (8.0%) carried a mutation at exon 9 of CALR gene (CALR+), 3 (2.02%) carried a mutation at codon 515 of MPL gene, 26 (17.58%) patients were not mutated for JAK2, CALR and MPL genes (triple-negative), 31 (21.01%) patients had one (CALR+, MPL+ or JAK2+), 29 (19.47%) patients had two (CALR+, MPL+ or JAK2+) and 15 (10.13%) patients had all three mutations (triple positive).

CALR+ patients had a younger age at diagnosis: median 48 years (25-92) in CALR+ patients vs 72 years (18-93), respectively. Patients with MPL mutation had a median age of 82 years while triple-negative 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 CALR+, 24% (15/62) in JAK2+ group, 18% (5/28) in MPL+ group and 30% (5/16) in the triple negative group. The IPSET1 model also stratified patients with statistically significant difference (p=0.001) among the three groups: the percentage of high-risk patients was 16%, 66 (22/13) in the CALR+ group, 82, 35% (88/107) in the JAK2+ group, and 33,33(8/29) in triple negative group. CALR+ patients belonged more frequently to the low intermediate risk group than JAK2+ patients (80% versus 17.5%, p=0.05). The incidence of thrombotic events at diagnosis of ET was 0 in the CALR+ group, 28.3% (30/107) in the JAK2+ group and 23.07% (6/26) in the triple negative group. The median overall survival was not reached in any group.

Conclusions: CALR+ patients with ET are phenotypically distinct from JAK2+ and triple negative patients. We can speculate a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSET1 high-risk patients.

PB2057

RUXOLITINIB IN MYELOFIBROSIS: A MULTICENTRE EXPERIENCE FROM THE EAST OF ENGLAND

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Background: Ruxolitinib, an oral Janus Kinase (JAK)1/JAK2 inhibitor, was approved in the EU in August 2012 for treating disease-related splenomegaly and constitutional symptoms in adults with primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (PPV-MF), and post-essential thrombocythemia 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 17 (38.6%). The most frequent non-haematologic AEs were minor infections, documented in 17 patients (38.6%), and included lower respiratory tract infections, candidiasis, and HSV/VZV reactivation. One patient died from Aspergillus pneumonia. Twenty-nine patients (65.9%) remain on treatment.

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.

PB2058

MONITORING OF TRANSIENT MYELOPROLIFERATIVE DISORDER AND LEUKEMIA IN DOWN’S SYNDROME: A SINGLE UNIVERSITY HOSPITAL STUDY

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Background: Children with Down syndrome (DS) have a 10- to 20-fold increased risk of developing leukemia. But some patients don’t suffer leukemia even they have significant numbers of blast cell in their peripheral blood. These patients differ from a disease condition called Transient myeloproliferative disorder (TMD), and it is a disease entity unique to DS newborns and is defined as the morphologic detection of blasts in DS less than three months of age.

Aims: This study gathered DS patients to find some difference between leukemia and TMD, to determine the threshold of blasts cell in their peripheral blood.

Methods: We collect 317 patient’s blood lab results in 433 DS patients. 102 patients had leukocytosis, and in 18 case found blast cells in their peripheral blood.

Results: 12 patients have found blast in three months of life, 11 of them finally diagnosed to TMD, and only 1 patient progress to Acute Myeloid Leukemia(AML) in 98 days of his life. Other 6 patients have blast in their blood after three months of life, and underwent chemotherapy due to hematologic malignancy. All patients with leukemia has anemia at diagnosis, which is not found in TMD patients.(p=0.018). In 7 Leukemia patients, 3 was acute Lymphoblastic leukemia and 4 was AML. All patients underwent chemotherapy additional to trisomy 21 at their diagnostic point, which didn't found at TMD and ALL patients, even it didn't confirm former examination.

Summary/Conclusions: DS Patient who has blast in their peripheral blood before 3 months of life need closely follow up their Complete Blood Count and Chromosome analysis to find whether TMD progress to leukemia.

PB2059

INFEKTIOUS EVENTS IN A COHORT OF PATIENTS WITH MYELOFIBROSIS UNDER TREATMENT COMPARING RUXOLITINIB WITH CONVENTIONAL THERAPY. A MONOCENTRIC EXPERIENCE OF 22 PATIENTS RETROSPECTIVELY ANALYSED

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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 predominately mediated by its profound anti-inflammatory effects modulating dendritic cell (DC) function resulting in impaired CD4+ and CD8+ activity. Several studies have shown that Ruxolitinib affects different cytokines (IL1, IL6 and TNFalpha) and other immune processes and has been linked to increased incidence of opportunistic and no opportunistic infections. Herein we report our experience at our Centre.

Aims: In our retrospective study we analysed myelofibrosis patients treated with Ruxolitinib and cytoreductive treatment with Hydroxyurea 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 Hydroxyurea taken orally, similar for age and clinical features.

Results: A total of 22 patients consecutively diagnosed were included in this analysis. There were 15 primary and 7 secondary myelofibrosis patients. According to the Dynamic International Prognostic Scoring System (DIPSS) 8 analysis. There were 15 primary and 7 secondary myelofibrosis patients. 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. Patients with ruxolitinib 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 and originates from a 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 cause 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 polycythemia 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 were 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 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% of patients with ET. Bleeding may mimic 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 (DIC).

**Aims:** The aim of this study is to monitor the count of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values as potential risk factors for the incidence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.

**Methods:** During the three-year period we monitored the occurrence of hemorrhagic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasms. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (61); 2. Group with essential thrombocythemia (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasms (MPNs) (25). The following possible risk factors were monitored: counts of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values. 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 4.5-8.8 x 10^12/L, leukocyte count 1.2-27.1 x 10^9/L and the platelet count ranged from 102-1886.6 x 10^9/L. Hemoglobin values ranged from 176-210 g/L, and hematocrit from 0.58 to 0.83 L/L. The highest erythrocyte count, the highest hemoglobin and hematocrit values, as well as the highest leukocyte count was recorded in the group of patients with PV and MPNs (p <0.001) and the lowest in the group of patients with IMF (p <0.01). Among the groups 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 erythrocytosis, but without statistical significance. The highest platelet count was found in the group of patients with ET and MPNs (p<0.001), and the lowest in the group of patients with IMF (p<0.01). Among the group of patients with PV and MPNs there was no statistically significant difference with regard to platelet count. Hemorrhagic complications were more frequent both in patients with platelet count below 10^10/L (p<0.05) and in patients with platelet count over 1000 x 10^9/L (p<0.01). The increase in platelet count influences the adsorption of larger von Willebrand multimers on the platelet membrane, thus having an effect on their elimination from circulation and degradation.

**Summary/Conclusions:** The platelet count can be considered a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Deviation from the count of leukocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasms, but further follow-up and a larger number of subjects are needed. The age of the patient can also be considered as a risk factor for the incidence of hemorrhagic syndrome in those patients. The follow-up of patients with unclassified myeloproliferative neoplasms has been particularly important, which showed a high prevalence of hemorrhagic complications, and with the purpose of their further differentiation.

**PB2062**

**CLINICAL RELEVANCE OF JAK2V617F MUTATIONAL LOAD IN PATIENTS WITH PHILIPPIALED MYELOPROLIFERATIVE NEOPLASMS FROM REPUBLIC OF MACEDONIA (SINGLE-CENTER EXPERIENCE)**

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**Background:** Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative neoplasms (MPNs); our experience suggests that ruxolitinib use in CNL patients can induce partial responses by improving marrow function and reducing in spleen size (209x119x74 mm). The platelet count can be considered a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Deviation from the count of leukocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasms, but further follow-up and a larger number of subjects are needed. The age of the patient can also be considered as a risk factor for the incidence of hemorrhagic syndrome in those patients. The follow-up of patients with unclassified myeloproliferative neoplasms has been particularly important, which showed a high prevalence of hemorrhagic complications, and with the purpose of their further differentiation.

**Aims:** The aim of this study is to monitor the count of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values as potential risk factors for the incidence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.

**Methods:** During the three-year period we monitored the occurrence of hemorrhagic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasms. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (61); 2. Group with essential thrombocythemia (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasms (MPNs) (25). The following possible risk factors were monitored: counts of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values. 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 4.5-8.8 x 10^12/L, leukocyte count 1.2-27.1 x 10^9/L and the platelet count ranged from 102-1886.6 x 10^9/L. Hemoglobin values ranged from 176-210 g/L, and hematocrit from 0.58 to 0.83 L/L. The highest erythrocyte count, the highest hemoglobin and hematocrit values, as well as the highest leukocyte count was recorded in the group of patients with PV and MPNs (p<0.001) and the lowest in the group of patients with IMF (p<0.01). Among the groups 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 erythrocytosis, but without statistical significance. The highest platelet count was found in the group of patients with ET and MPNs (p<0.001), and the lowest in the group of patients with IMF (p<0.01). Among the group of patients with PV and MPNs there was no statistically significant difference with regard to platelet count. Hemorrhagic complications were more frequent both in patients with platelet count below 10^10/L (p<0.05) and in patients with platelet count over 1000 x 10^9/L (p<0.01). The increase in platelet count influences the adsorption of larger von Willebrand multimers on the platelet membrane, thus having an effect on their elimination from circulation and degradation.

**Summary/Conclusions:** The platelet count can be considered a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Deviation from the count of leukocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasms, but further follow-up and a larger number of subjects are needed. The age of the patient can also be considered as a risk factor for the incidence of hemorrhagic syndrome in those patients. The follow-up of patients with unclassified myeloproliferative neoplasms has been particularly important, which showed a high prevalence of hemorrhagic complications, and with the purpose of their further differentiation.
Non-Hodgkin & Hodgkin lymphoma - Biology

PB2064
PERIPHERAL BLOOD CELL STUDY FROM PATIENTS WITH FOLLICULAR LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA: WHAT SHOULD WE EXPECT?
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Background: Follicular lymphoma (FL) may evolve to diffuse large B-cell lymphoma (DLBCL) and interactions between neoplastic cells and immune tumour microenvironment have been involved in this process. However, the potential value of the peripheral blood study to identify FL patients at high risk of progression is less known.

Aims: To describe the peripheral blood findings of patients with FL and DLBCL at diagnosis, and to investigate whether a particular lymphoid distribution could be associated with aggressive disease.

Methods: The study (performed between September 2012 and January 2017) included 52 patients (50% female) with a median age of 70.5 years (71% ≥60 years). Patients were newly diagnosed with in situ FL (n=1), Grade 1 FL (n=12), Grade 3 FL (n=11), and DLBCL not otherwise specified (n=28). In situ FL and Grade 1 FL were grouped as low-grade FL. Most patients with FL (11/13, low grade in situ and SLL/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 staining protocol and monoclonal antibodies. 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/UL of monocytic, 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%). 100% of cases had a low number of polyclonal B cells (<100/UL). Comparison of low-grade FL, grade 3 FL and DLBCL in low showed any statistically significant difference regarding monocytic, CD4, CD8 and total T cells. Low-grade FL and DLBCL showed the highest number of differences, involving lymphocytes (257±2439 versus 1495±671, p=0.001), NK cells (381±312 versus 204±167, p=0.03), the CD4:CD8 ratio (1.5±0.49 versus 2.06±1.44, p=0.002), and circulating mononuclear B cells, for both comparisons (0.25±0.17 versus 0.04±0.01, p<0.001) and absolute number (869±1758 versus 18.75±46.7, 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 percentage of expression of monoclonal B cells was higher in low-grade FL than in grade 3 FL, in both percentage (15.2±23.23 versus 1.94±23, p=0.001) and absolute number (66±41 versus 105±102, p=0.048).

Summary/Conclusions: The peripheral lymphocyte profile in patients with FL and DLBCL is heterogeneous, but low-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 MANTEL 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 PDK1 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 analysis (IHC) staining of phospho- (p-) PDK1 (Ser241). Cell proliferation was assessed by a modified MTT assay. Antibodies utilized for Western blotting was performed for evaluating protein expression levels of PDK1, p-PDK1(1Ser241), p-RSK2(Ser227), and RSK2. BX-912, a specific inhibitor for PDK1, was purchased from Selleckchem (USA). RNA interference of PDK1 was performed by transfection of small interfering RNA (siRNA) into MCL cell lines by means of nucleofection (Lonza, Switzerland). This study was approved by the institutional review board of our institute.

Results: By means of IHC examination, our study revealed that PDK1 was activated through phosphorylation in tumor cells of all 7 MCL patient-derived specimens examined, and this was also the case in all 5 MCL cell lines examined and in all 5 follicular lymphomas examined. These indicated that PDK1 is generally active in various types of B-cell lymphoid neoplasms. The in vitro treatment with BX-912 for 48 hours resulted in the dose-dependent inhibition of cell proliferation in all 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) and a BL cell line (IC50 2.9 mM). In addition, the flow cytometric analysis revealed that the growth inhibition of MCL cells by PDK1 blockade with BX-912 was at least partly mediated through the induction of apoptosis. As the molecular sequelae, PDK1 blockade by BX-912 resulted in dephosphorylation of RSK2 and AKT activity or CCND1 expression was unaltered by BX-912 treatment in MCL cells. By gene knockdown of PDK1 by RNA interference using three different short hairpin RNAs, we further validated that the reduction of PDK1 protein caused the inactivation of RSK2 and the growth inhibition in MCL cell lines. Finally, when combined with various agents those for the peripheral expression of monoclonal B cell, 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 PDK1/RSK2 signaling axis is the potential therapeutic target in MCL.
gested that KPUM-YY1R cells harbored the distinct gene expression patterns in MCL, a gene for p-glycoprotein (P-gp) of drug transporter micromolecular MGST1, a member of glutathione S-transferase (GST) families, and argininosuccinate synthetase 1 (ASS1), a rate-limiting enzyme for arginine biosynthesis. The upregulation of MDR1 (P-gp) and MGST1 were confirmed by Western blot or RT-PCR analysis in KPUM-YY1R compared with KPUM-YY1. Importantly, the addition of Pgp inhibitor, such as ethacrynic acid, at least partly restored the sensitivity to BH in KPUM-YY1R cells, indicating the functional significance of the upregulation of MDR1 and MGST1 in the development of BH resistance in MCL. In addition, BH-resistance cells were also found to express decreased mRNA level of ASS1 whose over expression has been reported to play tumor suppressor roles and its loss has been associated with clinical aggressiveness in various cancers.

Summary/Conclusions: This study revealed that the multiple molecular mechanisms overlappingly underlie the development of BH resistance, therefore, the acquisition of BH resistance potentially leads multidrug resistance in MCL cells. Thus, development of KPUM-YY1 cells and KPUM-YY1R cells deserve the identification of multiplex mechanisms underlying BH activity/resistance and the future development of strategy which overcomes the treatment refractoriness in MCL.

PB2067

COMPARISON OF OVERALL SURVIVAL ACCORDING TO BONE MARROW ASPIRATION RESULTS IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA

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Background: Bone marrow (BM) biopsy with or without aspiration is usually included in the staging workup for patients with non-Hodgkin’s lymphoma (NHL). According to the National Comprehensive Cancer Network guidelines, BM biopsy is mandatory for lymphoma, but aspiration is optional. Moreover, the role of BM aspiration is controversial. Other studies have shown that BM aspiration morphologically or flow cytometry is often inconsistent with biopsy and is less likely to detect lymphoma than biopsy. There are no clear guidelines regarding results that are positive in BM aspiration and negative in biopsy.

Aims: The aim of this study was to establish guidelines through a comparison of the overall survival (OS) of patients with NHL using morphological method.

Methods: We performed a retrospective analysis of BM involvement in patients with newly diagnosed NHL in the Korea University Hospital from January 1991 to December 2016. OS was compared according to the BM groups, which were divided into three groups: the group without BM involvement in both BM aspiration and biopsy, the group with atypical lymphocytes only in BM aspiration, and the group with BM involvement in biopsy regardless of BM aspiration results. Atypical lymphocytes were identified as positive in BM aspiration if they displayed cleaved nuclei, vacuolation both in aspiration and biopsy including lymphoid aggregates, 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.

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 (log-rank test, P<0.001). When comparing between 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).

Multivariate analysis was performed by adjusting survival related variables such as sex, age, lactate dehydrogenase, Ann Arbor stage, Eastern Cooperative Oncology Group performance status, number of extranodal sites, lymphoma type, Rai stage (0, low vs aggressive), and transplantations. The classification according to BM involvement remained a significant prognostic factor for OS (P<0.001). However, in the subgroup analysis, the group with atypical lymphocytes only in BM aspiration showed no significant difference compared to the group without the BM involvement in both BM aspiration and biopsy (Odds ratio, 1.915; 95% confidence interval, 0.940–3.903; P=0.074). Therefore, the detection of atypical lymphocytes only in BM aspiration had no significant difference in the OS even when the relevant factors were corrected.

Summary/Conclusions: This study suggests that the detection of morphologically atypical lymphocytes only in BM aspiration, but not in biopsy, is not significantly in predicting the OS of patients. Therefore, even if atypical lymphocytes are detected during BM aspiration in patients with NHL, it may not be sufficient to judge the BM involvement and predict the OS of these patients.

PB2068

IN VIVO IMAGING OF LUMINESCENT DIFFUSE LARGE B-CELL LYMPHOMA XENOGRAGTS COMBINED WITH MASS SPECTROMETRY IMAGING IDENTIFY SPECIFIC MOLECULAR ALTERATION DURING R-CHOP RELAPSE.

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin’s lymphoma (NHL) throughout the world, comprising 30–35% of all NHLs, with approximately 71 000 new cases and 19 000 deaths estimated for 2014. Currently, R-CHOP, a combination of immunotherapy (Rituximab, targeting the cell surface protein CD20 expressed by B cell lymphoma) and chemotherapy (Cyclophosphamide, doxorubicin, vincristine and prednisone), remains the most commonly used regimen 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 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 identify novel targets and drugs resistant to R-CHOP.

Aims: Our aim is to investigate and analyze the various chemical composition of DLBCL xenografts during tumoral development and R-CHOP treatment relapse, in order to identify yet uncharacterized targets that could become alternative targets for therapy.

Methods: 10 millions cells of a U2932 lymphoma cell line were xenografted into 60 athymic nude immuno-deficient mice. Tumoral growth was repeatedly quantified in a non-invasive manner based on tumors’ luminescent signal using the in vivo imaging system (IVIS) Lumina II. R-CHOP treatment was applied to mice after primary tumoral growth. 2 types of samples are generated: i) study of the therapy resistant and sensitive areas of each tumor.

Aims: The in vivo imaging allows us not only to precisely assess primary tumor
development but more importantly, to monitor accurately response to R-CHOP and relapse from this therapy. The tumors at different stages of response to R-CHOP therapy are being analyzed and compared from lipodisorders, metabolomics and proteomics point of view. Primary analysis indicate very distinctive metabolomics and lipodisomics fingerprints between relapsed and non treated tumors.

Summary/Conclusions: Combinig 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 lipodisomics, 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: Indoleamnine 2,3-dioxygenase (IDO) is an inducible enzyme that catalyzes the initial and rate-limiting step in tryptophan along the kynurenic 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 in hematological malignancies. We are currently investigating in more details the 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/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 between histological subtypes of HL. We also did not find any association between stage and IDO expression in our study. Patients with the absence of IDO expression tended to have a better response to the 1st line chemotherapy comparing to patients with positive IDO expression. The overall response rate was achieved in 71.4% (5/7) of IDO+ cases and in 92.9% (26/28) of IDO− cases. The relapse or tumor progression on treatment was observed in 57.1% (4/7) of IDO+ cases and in 10.7% (3/28) of IDO− cases. Secondary chromosomal abnormalities were detected in 40 (60%) patients, with 5 or more abnormalities in 4 patients, 3 chromosomal abnormalities in 2 patients, and 2 chromosomal abnormalities in 20 patients. The most 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 translocation (6 patients), chromosome 6q deletion (4 patients), chromosome 13q deletion (3 patients), marker chromosome (3 patients), loss of chromosome 17p21 (2 patients), isochromosome 9q (2 patients), translocation of chromosome 13, trisomy 13 and trisomy 9 in one patients each. Relapse or tumor progression on chemotherapy was seen in 16% of the whole group of patients. The 5 year OS was 57.7%, while 5 year EFS was 51.6%. When comparing incidence of relapse in patients with complex karyotype, we found that nine out of 16 (56.2%) patients having complex karyotype experienced relapse whereas relapse occurred in only 6 (12.5%) patients having non-complex karyotype (p-value= 0.005)

Summary/Conclusions: The frequency of secondary chromosomal abnor- malities in our series is in concordance with other publications with duplication 1q being the most common, followed by deletion 13q, 1q, and 17p. Complex karyotype was significantly associated with higher incidence of relapse and poor outcome.

PB2071

IGVH 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 incident clinical course and a non-characteristic phenotype and karyotypic abnormality. SMZL and SDRPL are two separate cell lymphoma and characterized by a peculiar morphology with micronodular pattern of infiltration, biphasic cytology, and the almost constant presence of marginal zone differentiation. Splenic diffuse red pulp lymphoma been introduced as a provisional entity but differential diagnosis with other SLs is needed to be exact and to choose the therapeutic strategy. In the present study we aimed to compare the VH genes in both cell lymphoma.

Aims: The aim of our study to determine the immunoglobulin variable heavy chain (IgVH) gene usage and somatic mutation patterns in a series of SMZL and SDRP patients.

Methods: We studied 24 patients with SMZL, 40 patients with HCL and 10 patients with SDRPL. Diagnosis was based on standard Western criteria. 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 IgVH genes were amplified essentially in reactions that contained only one of the 5’ leader region primers for the indicated VH gene family. All PCR reactions were performed using appropriate positive and negative controls. The rearranged VH genes identified for each case seemed to represent functional rearrangements because no stop codons or crippling mutations were identified.

Results: A comparison of the VH genes to reported germline sequences in SMZL reveals that 4 cases use VH1 family, 1 case uses VH4 family, 16 the VH1 family segments. All cases were determined by translocations involving the MYC gene to one of the immunoglobulin genes. The clinical significance of secondary chromosomal abnormalities associated with this characterisation translocation remains unknown.
CELL OF ORIGIN ASSIGNMENT USING IMMUNOHISTOCHEMISTRY IS INFLUENCED BY BCL-2 EXPRESSION IN DLBCL PATIENTS TREATED WITH CHEMO-IMMUNOTHERAPY

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Background: Epstein–Barr virus (EBV) infects more than 90% of the population worldwide. The virus has evolved to persist-long in B-lymphocytes of infected individuals, but disruption of this tightly regulated B-cell infection could result in EBV-associated B cell lymphomas. In Argentina, primary infection is mostly subclinical and 90% of patients are seropositive by 3 years old. However, EBV presence is statistically associated with Hodgkin lymphoma (HL) and diffuse large B-cell lymphoma (DLBCL) in patients younger than 10 years, suggesting a relationship between EBV infection and B-cell lymphoma development in children from Argentina.

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 combinational chemotherapy containing rituximab. Patients who died prior to receiving therapy were excluded. Categorical and continuous variables were compared using Chi-squared and Wilcoxon tests, respectively. Time to event 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 performed 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 56.2% respectively (Figure 1). Interestingly, BCL-2 expression predicted OS irrespective of COO assignment. Patients with BCL-2 expression had a 2-year OS of 55.6% vs 56.2% for GCB and non-GCB, respectively. Whereas, patients without BCL-2 expression had a superior 2-year OS at 79.9% vs 78.3% for GCB and non-GCB, respectively (p=0.02).

Summary/Conclusions: COO assignment using IHC demonstrated superior PFS for GCB over non-GCB however this was mitigated by BCL-2 expression. This raises questions regarding the currently presumed pathogenesis of the different subtypes and how to utilize the currently available targeted therapies including BCL-2 inhibitors. These observations warrant further study.

PB2072

DIFFUSE LARGE B-CELL LYMPHOMAS AND CARRIERS REGARDING LATENCY PROFILE AND MICROENVIRONMENT COMPOSITION INVOLVED IN LYMPHOMAGENESIS?

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Background: Epstein–Barr virus (EBV) infects more than 90% of the population worldwide. The virus has evolved to persist-long in B-lymphocytes of infected individuals, but disruption of this tightly regulated B-cell infection could result in EBV-associated B cell lymphomas. In Argentina, primary infection is mostly subclinical and 90% of patients are seropositive by 3 years old. However, EBV presence is statistically associated with Hodgkin lymphoma (HL) and Diffuse Large B-cell lymphoma (DLBCL) in patients younger than 10 years, suggesting a relationship between EBV infection and B-cell lymphoma development in children from Argentina.

Aims: Given that viral latent proteins and microenvironment composition play a key role in tumor pathogenesis or control of viral infection, our aim was to compare this scenario in pediatric EBV-associated lymphomas derived from the germinal center (GC) and post-GC 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 counts were higher in EBV+ non-GC carriers, whereas CD8+ cell counts were significantly lower in EBV+ GC pediatric carriers (p=0.014, Mann Whitney test), whereas statistically higher CD4+ cells were counted at the EBV+ GC in pediatric carriers (p=0.004, Mann Whitney test). On the other hand, CD8+ cells did not exhibit statistical differences neither in EBV-associated lymphomas nor in benign conditions at the GC, and the same was observed for CD4+ helper (CD4+) cells (p=0.005, Mann Whitney test). In contrast, CD4+ cell count were statistically higher exclusively at EBV+ subepithelial region in tonsils, compared to EBV- counterpart (p=0.0039, Mann Whitney test). Finally, cytotoxic activity evaluated by GrB expression displayed a trend to higher mean in EBV+ DLBCL (p=0.057, Mann Whitney test) but no in HL. Concerning EBV, pediatric carriers did not show differences in cytotoxic activity according to EBV presence at the GC (p>0.05, Mann Whitney test). In fact, GrB cytotoxic activity was prevalent only at the EBV+ subepithelial region (p=0.0420, Mann Whitney test).

Summary/Conclusions: Latency II pattern prevails in both pediatric EBV-associated lymphomas and in EBV+- GC 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+ helper cell response plays a key role at the GC region in EBV carriers, by participating directly as effectors cells, by helping to the overall immune response in the control of viral infection and restrict latency expression to type II pattern, and, ultimately, by limiting the cell outgrowth. Failure in this process may trigger malignant transformation in EBV-associated lymphomas.

PB2074

MICROARRAY EXPRESSION PROFILE OF LONG NONCODING RNAS IN GERMINAL CENTER-LIKE DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Long noncoding RNAs (lncRNAs) are constantly transcribed and involved in a variety of biological activities. The contributions of lncRNAs 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 lncRNAs 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 lncRNAs 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 IncRNAs in GCB DLBCL.

**Results:** We demonstrated that 21,539 IncRNAs were expressed in all samples analyzed, of which 1,548 IncRNAs were upregulated and 2,671 IncRNAs were downregulated in GCB DLBCL cell lines (OCI-ly1 and OCI-ly19) (≥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 IncRNA-mRNA co-expression network was constructed to identify potential target genes related to the 3 upregulated and 2 downregulated IncRNAs.

**Summary/Conclusions:** Our data suggested that IncRNAs may play an important role in the pathogenesis of GCB DLBCL, and profile of IncRNAs may be used as a potential biomarker in the diagnosis of DLBCL and predicting its clinical outcome.

**Keywords:** Flow cytometry, extranodal lymphoma

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**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. Flow cytometry at extranodal sites can 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 served 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 along with cytological findings were compared with histological and IHC diagnosis.

**Results:** Flowcytometric immunophenotyping conducted on extranodal sites included total 10/45 (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), ALC (1 case), Mantle cell lymphoma (1 case) and Ewing’s/ENET (1 case). Combining FCI with pathological findings definite diagnosis could be found in 33/40 (82%) cases compared to 30/40 (75%) in extranodal samples. Pathological evaluation samples collected in isolation were submitted for FCI on 5-color Beckman Coulter FC-500, using a set of mature and immunosuppressive adenosine. Several studies have demonstrated in humans the overexpression of CD39 (NTPDase) and low adenosine deaminase (ADA) 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 analysed by flow cytometry (FC) the T CD4 lymphocytes of solid biopsies, the surrounding hematopoietic cells in Hodgkin lymphoma (HL) lymph nodes and we demonstrated the presence of an activated profile (CD39+) with a reduction of CD26 (CD4+CD26-CD39+). We also confirmed a link between a activated environment (CD39+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The profile by FC of CD4 T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target.

**Aims:** We wanted to test if this subset may also characterize T infiltrating lymphocytes throughout 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+ cells is confirmed. Compared with HL, the cells of DLBCL are not statistically (Student 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 DLBCL.

**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 (CD39+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The profile by FC of CD4 T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target.

**Keywords:** Flow cytometry, extranodal lymphoma
the clone of B lymphocytes involved in cancer. This may support that leukemic cells may contribute to create and characterize an immune-subversive environment and to facilitate immune escape mechanisms. FC analysis of CD26 and CD39, markers likely connected with the adenosinergic pathway, in PB can represent effective parameters to determine and characterize the Treg CD4 in different types of lymphoma and could serve as targets in the follow-up of HL and B-NHL.

PB2078

BCL-2 AND Ki-67 AS INDEPENDENT PREDICTORS OF POOR-RISK IPI GROUP OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is heterogeneous disease in terms of clinical behaviour, morphology, phenotype and genetics. Gene expression profiling has made a distinction between two entities germinal center B-phenotype (GC), activated B-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. The 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 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, biological and immunohistochemical data have been studied. Use of CL and biomarkers individually. The impact of the expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI-highest score in multiple regression analysis, afterwards in regression equation and variance analysis.

Results: Group with GCB phenotype (defined by expression of bcl-2, bcl-6, CD10 and MUM1) according to Hans’s and Muris’s algorithm showed positive correlation with good-risk patients identified by IPI. Multiple regression analysis showed that bcl-2, bcl-6, CD10, MUM1 and Ki67 on IPI had a significant impact.

Summary/Conclusions: Multiple regression analysis proved impact of biomarkers on IPI. Following this analysis, bcl-2 and Ki67 are independent predictors of poor-risk IPI group of patients, (bcl-2: p = 0.0107, Ki67: p = 0.0377). The value of F-ratio 2.9845 proves that there is a linear connection between models including all variables bcl-2, bcl-6, CD10, MUM1 and variables depend on the value (IPI) (p = 0.0210). The mutual impact of bcl-2, bcl-6, MUM1, Ki67 is significantly related to poor-risk IPI patients.

PB2079

COMPARATIVE PATHOLOGIC ANALYSIS OF MEDIASTINAL B-CELL LYMPHOMAS: EXPRESSION OF P63 BEST DIFFERENTIATES PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA FROM CLASSICAL HODGKIN LYMPHOMA


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Background: Mature B-cell lymphomas of the mediastinum include primary mediastinal large B-cell lymphoma (PMLBCL), classic Hodgkin Lymphoma (CHL), B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and CHL (subtype mediastinal) and others. PMLBCL and mediastinal CHL, mostly nodular sclerosis (NS) share many clinicopathologic characteristics, however, therapeutic options and responses are quite different.

Aims: We aimed to find distinct 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 reticulin 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 microvessel density and the subtype of the disease has not been established yet. Our aim is to investigate the association between microvessel density and histologic 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 were selected. The density of blood vessels in CD was based on general preserved immunohistoarchitecture with typical angio-follicular hyperplasia, circular arrangement of mantle cells around hyalinized germinal centers (“onion skin” pattern). The plasma cell type of CD was confirmed by presence of perifollicular sheets of CD138+ plasma cells. Vessels were counted by digital images with CD34 staining. Slides were scanned on the whole slide digital Panoramic scanner. Percentage of blood vessel area (vessel density index) was calculated using Panoramic Viewer software, statistical analysis was conducted with Student’s-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% (mean ± standard deviation) of the area. In patients with plasma cell variant percentage of blood vessel area was increased to 15±1.4% (p<0.05). Patients with hyaline vascular CD were divided into 2 groups depending on the vessel density index. In 15 patients (75%) percentage of vessel area was 6.8±2.3%, which was somewhat lower than in patients with plasma cell variant (not statistically significant). In 5 patients (25%) with hyaline vascular CD, the percentage of vessel area was higher - 12±3.1%, (p<0.05) and did not differ from levels in patients with plasma cell variant.

Summary/Conclusions: The highest index of vessel density in the lymph node was observed in the plasma cell variant of CD. However, 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.
Aim: 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 a male/female ratio of 1.3/1. Ninety-one patients were eligible for R-CHOP treatment. The median follow-up was 41 months. Out of the 90 cases analyzed by immunohistochemistry CD 10, BCL2, BCL6 and MUM1 expression was found in 17.6%, 50.5%, 72.7% and 81.8% of cases, respectively. Negative expression for CD10, as well as positive expression of BCL2, were adverse prognostic factors for 3-years overall survival (OS) and disease free survival (DFS) (OS for bcl2: 72.3 vs 89.7, p<0.05, OS for CD10: 84.1 vs 75.1, p<0.05). BCL6 and MUM1 expressions, however, did influence neither OS nor DFS.

Summary/Conclusions: This study confirms the prognostic value of a multi-marker assessment which includes bcl2, bcl6, CD 10 and MUM1 expression for patients R-CHOP therapy.

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, hypertriglyceridemia and/or coagulopathy. Histiocyte Society (HS) criteria have been applied for diagnosing HLH, however not all of them are usually shown 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.
Summary/Conclusions: THLH triggered by LN is diagnosed in older patients than other causes secondary HLH (46-80 vs 4-8 y/o in our center), we think this is because in our experience there are not children or Young adult in HLH due to LN group. We would like to highlight that although LN is a very common HLH trigger there are a few works describing them in the literature, that is why we would like to spread our experience. We would like to emphasize in the importance of early diagnosis. Despite being a rare disease, it is still under diagnosed, reaching the diagnosis most of the times after seeing hemolytic anaemia phenomena in bone marrow biopsy. Agreeing with literature, main consulting reasons are similar to our series. Correlation between neoplastic activity and immune activation, as well as test and facts which could predict evolution should be more studied. Finally we would like to address the necessity of considering this possibility in the face of a patient with fever which does not respond to antibiotics and has not clarified citopenia and also the importance of conducting cheap and very profitable test such as ferritin or tryptophan 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. These networks are put in place to facilitate 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 centres and/or research laboratories, 9 patients’ associations on behalf of 7 scientific societies.

Results: The main missions of this network are to improve the care, the research and to educate professionals, patients as well as to disseminate more information and recommendations 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 links between clinical and epidemiological surveillance. The research project manager watch out for calls for tender, set up new registers and continually monitor the regulations for retrospective and prospective studies, both in France and at the international level. Furthermore, MaRIH supported successfully the application of several of its members for European reference networks (Figure 1).

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 reaction to therapy, set-up of new registries and continually monitor the regulations for retrospective and prospective studies, both in France and in other countries can have easily an expert opinion for their patients. At the same time, improving the child-adult transition was identified by the steering committee as a top priority. Communication and training: MaRIH is involved in organizing many events in France to improve the visibility of the centres and to provide education on these rare diseases. The 1st annual conference of the network took place on June 25th 2015 and the third one is planned on June 1st 2017 in Paris. Moreover, a patient’s day meeting was organized on January 30th 2016 in Paris to inform on the update status of research on their disease as well as to help patients in daily common problems (sport, psychological, transfusion…). Pushing forward research development and epidemiological surveillance: the network has appointed a research project manager for its scientific and strategic committee to support, provide stability for and add value to research centre activities. The research project manager watch out for calls for tender, set up new registers and continually monitor the regulations for retrospective and prospective studies, both in France and at the international level. Furthermore, MaRIH supported successfully the application of several of its members for European reference networks (Figure 1).

Figure 1.
Summary/Conclusions: The creation of these new networks allows strengthening the links between the various actors involved in the field to improve care and answer transversal questions. In this way, MaRIH 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.
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 epis-taxis and sepsis. He had chronic TTP diagnosis for two years ago. We diag-nosed the patient as relapse TTP. Early treatment against infection and PLEX increased his platelet counts as early as the second day of treatment. The sixth patient presented with a fever that had been going on for five days. We treated the patient with PLEX together with the corticosteroid. Because his ADAMTS13 level was very low and he had 35% schistocytes.

Table 1.

Summary/Conclusions: We diagnosed our first patient with carcinoma unknown primary, who did not respond to PLEX and corticosteroid treatment. The results we received for that patient indicate that PLEX with corticosteroid treatment alone, remain ineffective in cancer-related TMA patients. Etiology of our second patients TMA was idiopathic. His clinical and laboratory findings improved rapidly in response to PLEX and pulse corticosteroid treatment. One viral infection induced TMA patient had anaphylactic reaction receiving his 8th PLEX. Allergic reactions should always be kept in mind when administering PLEX. One patient with TMA and malignant hypertension-induced renal failure was successfully treated with PLEX, hemodialysis and antihypertensive treatment. We successfully treated our bacterial infection and sepsis-induced TTP patients with PLEX and antibiotic administration. In second TMA patient, we coupled PLEX with high dosage corticosteroid treatment even though he had an infection. For he had high histocytocyte count and atypical neurological find-ings. 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 TS13 defi-ciency is the major cause in acquired TTP, finding the etiology of other TMAs is determinant for a successful treatment of the latter.

PB2086

CLINICAL SIGNIFICANCE OF ELEVATED SERUM COBALAMIN (VITAMIN B12) LEVELS

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Background: Hypercobalaminemia is a frequent but underestimated abnor-mality. Elevated serum cobalamin levels may be a sign of a wide range of dis-eases like solid and neoplasms, hematological disorders like blood and platelet disorders, chronic myelogenous leukemia, promyelocytic leukemia, poly-cythemia vera, hyer eosinophilic syndrome as well as liver and kidney dis-eases.

Aims: We aimed to evaluate the underlying disorders of the patients with high cobalamine levels (>1000 pmol/l) between 01.02.2016- 01.02.2017 in Hacette-pe University Pediatric Hematology Department.

Methods: We investigated the patient records of the patients examined between 01.02.2016- 01.02.2017 in our department and included the patients with serum cobalamine levels higher than 1000 pmol/l. We excluded the patients who are taking Vitamin B12 supplement.

Results: There were 46 patients with serum cobalamine levels higher than 1000 pmol/l out of 14367 patients seen between 01.02.2016- 01.02.2017 in our department. The reason to check the cobalamin levels were anemia, neu-tropenia and thrombocytopenia in most of the patients. Only 2 patients were referred to our department because of hypercobalaminemia. The underlying disorders were found to be leukemia in 3 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, bicytopenia in 4 patients and aplastic anemia in 2 patients, cobalamin metabolism defects in 10 patients, hypereosinophilia in 2 patients, polisitemia in 1 patient, cystic fibrosis in 1 patient, HIV in 1 patient, FFM (familial mediterrenian fever) in 1 patient, chronic kidney failure in 2 patients, sickle cell anemia in 1 patient, factor V Leiden in 1 patient, and hypertension in 1 patient. Overall, we found 28 different etiologies in 41 patients (93.5%) and cobalamine deficiency 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 clues. The determination of hematological findings has a positive effect on the prognosis of metabolic diseases.

Aims: The aim of this study is to evaluate the incidence of hematological find-ings in inherited metabolic diseases since there are a few studies about the true incidence in literature.

Methods: Three hundred eighteen patients who were being followed-up within the previous 6 months at Gazi University Department of Pediatric Nutrition and Metabolism, Turkey, were included in the study. Patients’ hematological findings were taken from Department of Pediatric Nutrition and Metabolism and hospital data-processing records. Since patients were in different age groups, hematolog-ical findings were compared with normal values for each patient’s age group. The hematological findings were classified under seven main groups; anemia, cytopenia and hemostasis disorders, chronic myelogeneous leukemia, promyelocytic leukemia, poly-cythemia vera, hyper eosinophilic syndrome as well as liver and kidney dis-eases.

Aims: To evaluate the link between LSDs and haemolysis and if it could be used as a screening test in LSDs.

Methods: The osmotic resistance test (ORT) was evaluated in 150 samples including controls, LSDs carriers (LSDs-C) and LSDs patients (LSDs-P). Briefly, the blood was mixed with different concentrations of sodium chloride solution (NaCl) and the haemoglobin released was quantified by spectrophotometry. The raw data was normalized using isonicotinic solution (0.9% NaCl). The statistical analysis (non-parametric tests and ROC curves), was computed by IBM SPSS stats 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=0.729) where no significant dif-fferences 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.
Results:

HemosIL SynthA Fox-SF which was sensitive against only lupus anticoagulant. It contained colloidal silica and normal range of APTT-SP was 25.4-36.9 s. Second reagent sensitive against both plasma factors and lupus anticoagulant. It contains mix of silica, ellagic acid and phospholipids by composed of syntethic or animal plasma. Doses 200 mg/kg/bw/day were defined and were the same in all studies. Blood samples for hematological measurements were examined at 4, 9, 13 weeks after TB exposure in the same groups of animals throughout the experiment. Hemoglobin (HGB) concentration, hematocrit (HCT), total amount of erythrocytes (RBC), leukocytes (WBC) and platelets (PLT), mean corpuscular hemoglobin (MCH) were evaluated.

Results:

As a result, all generic TBs on high toxic doses level (200 mg/kg/bw/day) had shown the tendencies for quantitative hematological changes. TBs mainly provoked the significant decrease of HGB concentration and RBC count on 4th and 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 induced anemia. The leukocytes count in peripheral blood. In case of generic pesticides, the presence of impurities can demonstrate various hematotoxic action. Also the adverse effects on peripheral blood of males Wistar Han rats were observed at a dose of 50 mg/kg/bw/day and demonstrated the lesions of red blood. But abovementioned changes were not so clearly expressed. Any adverse hematotoxic effects at 10 mg/kg/bw/day dose were not observed in all studies.

Summary/Conclusions:

As a conclusion, due to our results the triazole fungicides generic tebuconazoles have hematotoxic action. They induce anemia in Wistar Han rats and quantitative white blood cells changes. Today it is very important to investigate the hazardous effects of pesticides on the blood system.

Acquired pure red cell aplasia in an adolescent: could it be anything else?

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Background:

Pure red cell aplasia (PRCA) is a syndrome characterised by normocytic (sometimes macrocytic), normochromic anemia with severe reticuloctyopenia and marked reduction or absence of erythroid precursors from the bone marrow. PRCA may be congenital, in the form of Diamond-Blackfan Anemia, or acquired which is rather rare in childhood. An immune mechanism interrupting erythroid differentiation is responsible in primary autoimmune PRCA, on the other hand secondary acquired PRCA may be associated with autoimmune/collagen vascular disorders, infections, lymphoproliferative disorders, hematological malignancies, solid tumors and drugs.

Aims:

Here 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 congenital thrombocytopenia when he was 5 years old and the other reason since that time. There was no history of bone transfusion, chronic illness or any other medication. His physical examination revealed pallor and a 2/6 systolic murmur with no other abnormalities. His complete blood count revealed severe macrocytic anemia and reticuloctyopenia with hemoglobin:2.2 g/dL, hematocrit:1.2%, mean corpuscular volume:108.7 fl, red blood cell: 0.57x10^{12}/L, reticulocyte: 0.2% and mild leukopenia and lymphopenia. Peripheral blood smear showed macrocytic red cells with occasional tear drop cells. Stool for occult blood was negative. The direct and indirect antiglobulin tests were negative. Serum bilirubin, LDH, haptoglobin, liver function tests and renal function tests were in normal limits. Hemoglobin F was 2.9%. Bone marrow aspiration showed red cell hypoplasia, without dysplasia or giant pronormoblasts and normal myeloid and megakaryocytic series. A high resolution computed tomography of chest ruled out thymoma. Serum immunoglobulins revealed low IgA with normal IgG and IgM levels. Anti-nuclear antibody was positive and no autoantibodies were detected. Parvovirus B19 DNA were negative. Autoantibodies 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 a dose of 1 mg/kg/day. A diagnosis of AIDs was suspected and AIDs testing were started to maintain through levels of 150-250 ng/mL. His hemoglobin level gradually increased and reached to 12 g/dL and leukopenia and reticuloctyopenia resolved completely. Prednisone was tapered after 4 weeks and stopped. He is still on cyclosporine treatment and has been transference 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 lymphopenia, low immunoglobulin A level and positive anti-dsDNA in further investigations, yet these results are not sufficient for a specific diagnosis like common variable immunodeficiency syndrome, lupus anticoagulant syndrome. 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 severe anemia, 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 aplastic anemia 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 gastrointestinal blood loss accounts for 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 severe anemia, 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 aplastic anemia will further improve outcome and diminish the disease’s late complications.

Results: The median age was 62 (range 32 to 88) years old. 168 of 215 (78.5%) 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 (14.8%), 43 (20.0%), patients, respectively. FOBT was positive in only 65 (30.23%) subjects. 170 (79.06%) patients underwent 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 7 (7.90%) patients, colon polyp in 10 (4.56%) patients and hemorrhoid in 17 (7.99%) 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, 28,497 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=0.8221, 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 indices. 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 and/or 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 the North-Western region of Russia (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 (9 men and 14 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 -592CC genotype was slightly increased in the ITP group when compared to controls (65.7% vs 54.0% respectively; OR=1.6, 95% CI: 0.9-3.1, p=0.15). Interestingly, this variant of the IL-10 gene was more prevalent among women than men with ITP (71.2% vs 25.0% respectively; OR=7.4, 95% CI: 1.4-40.5, p=0.016). When compared to controls, the IL-10 -592CC genotype was significantly overrepresented in the group of women with ITP (71.2% vs 54.0%; OR=2.1, 95% CI: 1.1-4.2, p=0.044). On the contrary, in the group of affected men we observed the increase of persons who had IL-10 -592A allele (75.0% vs 46.0% in control group; OR=3.5, 95% CI: 0.7-18.3, p=0.15). Genotype frequencies for other studied genes were similar between the patients and control group as well as between women and men with ITP. We have also found almost 2-fold increase of the IL-1b -31CC frequency in women diagnosed before 30 years old compared to other patients (15.8% vs 8.2% respectively; OR=2.1, 95% CI: 0.4-10.5, p=0.39). The presence of the TNFA -308A allele was more often seen in patients diagnosed before 50 years old (26.7% vs 8.7% in other ITP patients; OR=3.8, 95% CI: 0.8-18.8, p=0.12).

Summary/Conclusions: We suggest that the IL-10 -592CC genotype is associated with increased risk of ITP 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.

Platelets disorders

PB2095
COMBINED TREATMENT OF AZATHIOPRINE AND ROMIPLOSTIM IN PATIENTS ITP REFRACTORY TO STEROIDS OR THROMBOPOIETIN ANALOGS
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Background: More than 70% of patients with Immune Primary Thrombocytopenia (ITP) respond to steroids, but 40 to 70% relapse in the first year follow-up. The use of romiplostim in this group is effective, although 8% failure has been described. In recent literature, there are clinical cases and small series describing the potentiating effect of combined treatment with thrombopoietin analogues and immunosuppressive drugs such as steroids, cyclophosphamide and rituximab. We have not found references to the combined use of azathioprine (AZA) and romiplostim (ROM).

Aims: To describe our experience in the combined use of azathioprine and romiplostim as a rescue treatment in patients with acute or newly diagnosed ITP refractory to corticosteroids or corticosteroid-dependence and refractory to maximal doses of romiplostim monotherapy.

Methods: We analyzed patients with newly diagnosed or persistent ITP, with corticosteroid-dependence or refractory to steroids and refractory to romiplostim, both in monotherapy. We have considered refractoriness to steroids not reaching platelets higher than 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 severe bleeding. We have considered refractoriness to romiplostim not get platelets greater than 30x10 9 /L with 10mcg/kg/week for at least 3 consecutive weeks. All patients have been diagnosed in a single center with the same physician responsible for the treatment and follow-up. The initial doses of AZA was 100mg/days (2mg/kg/day) and ROM 10mcg/kg/week. Patients have been evaluated every week until platelets were higher than 30x10 9 /L for to consecutive weeks, after this they were reviewed monthly.

Results: We treated 4 patients (75% female) with a median age at diagnosis of ITP of 53 years old (RIQ, 20-61 years). Treatments received prior to the use of the combination of AZA and ROM were polyclonal immunoglobulins (IG), cyclophosphamide and rituximab. Responses to steroids and romiplostim in monotherapy were: • Median dexamethasone cycles (40mg/days x 4 days) was 2.5 (2-4 cycles, IQR). The initial dose of prednisone was 1-2mg/kg/days with a median treatment day of 31.5 days (28-60 days, IQR). The type of response to steroids was PR with corticosteroid-pendence in one patient, 3 patients NR. • Median time from ITP diagnosis and romiplostim indication was 9.5 weeks (7-48 weeks, IQR). Median platelets 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 6 months of combined treatment and refractory to azathioprine. • One patient with RC maintains for 7 months in the absence of active treatment. The combination was necessary during 8 months. • 2 CRs still undergoing combined dose reduction (current dose romiplostim 2mcg/kg/week and azathioprine 50mg /d). Median platelets from onset of dose reduction 169x10 9 /L (128-176x10 9 /L, IQR). Duration of RC, 7 and 14 months.

Non adverse events have been described in combination treatment.

Summary/Conclusions: The use of azathioprine and romiplostim in combination could be a safe and effective alternative in subjects refractory to steroids or corticosteroid-dependent 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 oncological 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 hemato-oncological patients.

Methods: Inclusion was possible for admitted hematology-oncology patients aged 18 years and above after written informed consent. Furthermore, an expected need for platelet transfusions was necessary. Bleeding was graded according to the WHO bleeding scale. Platelet reactivity to stimulation by either adenosine diphosphate (ADP), crosslinked-collagen-related peptide (CRP-ΧL), PAR1- or PAR4-activating peptide (AP) was measured using flow cytometry.

Results: A total of 114 evaluations were available from 21 consecutive patients. Platelet reactivity in response to stimulation by all four studied agonists was inversely correlated with significant bleeding. Odds Ratio’s (OR) for bleeding were 0.28 for every unit increase in median fluorescence intensity (MFI) [95% Confidence interval (CI) 0.11-0.73] for ADP; 0.59 [0.40-0.87] for CRP-ΧL; 0.59 [0.37-0.94] for PAR1-AP and 0.43 [0.23-0.79] for PAR4-AP. The platelet count was not correlated with bleeding (OR 0.99 [0.96-1.02]).

Summary/Conclusions: The ex vivo 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 of the disease. The aim of the study might be to find the base for future specific immunomodulatory therapies for chronic ITP (cITP) in children.

Aims: The current case-control study aimed at detecting TNF-α (<308G/A) and TNF-β (<252A/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 heterozygous TNF-α genotype showed statistically significant higher mean age, longer disease duration & lower mean platelet count (p=0.005, 0.024 and 0.008 respectively). TNF-α polymorphism was more frequent among unresponsive patients compared to responsive patients with statistically significant difference. Calculated risk estimation revealed that combined genes polymorphism conferred three fold increased risk of development of cITP (OR=3.491, 95% CI: 1.235-9.869, p=0.015).

Summary/Conclusions: We hereby report a strong association between combined polymorphisms of both TNF-α & TNF-β genes and susceptibility to chronicity of ITP in Egyptian children. Further studies for gene polymorphisms which could affect the pathogenesis of ITP and facilitate the development of new therapeutic modalities are recommended.

PB2098

PROGNOSTIC FACTORS IN PRIMARY IMMUNE THROMBOCYTOPENIA OF CHILDHOOD

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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. New studies have yet to be 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 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 x10^9/L at diagnosis was found more frequently in newly diagnosed/persistent group (79% vs 36%, p=0.01). Similar, but not statistically significant difference, was found with mucosal bleedings (70% vs 50%, p=0.81). Children with newly diagnosed/persistent disease had less frequently impaired immunological markers (12% vs 65%, p=0.001) and received more 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 to chemotherapy or radiotherapy, not suggestive of bone marrow infiltration, drug-induced, infection of disseminated intravascular coagulation. For the diagnosis, we used an examination of bone marrow and number of children with IT was not mandatory.

Results: The two most common cancers associated with IT were bladder and lung neoplasms, but the occurrence of prior cancer (third part of patients) was not uncommon. The IT can appear at any stages of cancer, and it is mainly detected at the first two years after the diagnosis when the patient have been in acceptable antitumor 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:

PB2100
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: a) Neonatal sepsis (as defined by either culture positivity and/or clinical features of sepsis), b) Neonates 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 of 0.6 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).

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. Low 1, T. Dull 1
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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 <50x10^9/L at presentation however, National BCSH Guidance advises against platelet transfusion in TTP due to the perceived high aggregability state and risk of associated fatal thrombosis. The risk of thrombocytopenia related haemorrhage however 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 determine the average platelet count at time of line insertion and to determine 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 any significant bleeding complications were documented during or after line insertion. 5 patients had ‘excessive oozing at the insertion site’ documented, within the first 24 hours of insertion, for which no intervention was required. There were no deaths related to line insertion.

Summary/Conclusions: In conclusion, this study shows no significant bleeding risk associated with central venous catheter insertion in 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. Kalou1, E. Gavrilaki1,2, G. Papaioanou1, Z. Bousiou1, M. Iksa1, C. Vadikolios1, C. Lalayanis1, A. Athanasiadou1, R. Saloum1, A. Anagnostopoulou1
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Background: Management of chronic immune thrombocytopenia (cITP) aims not only to increase and maintain platelet counts in safe levels, but also to improve 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: Elnrombopag was administered in 15 pts, 6 male:9 female with a median age of 46 years (19-75 yrs) for 13 months (1-4.54 mo). Patients had received a median of 1 previous treatment (range 1-7); corticosteroids (15/15), intravenous immunoglobulin (5/15), rituximab (2/15), vincristine (1/15), cyclophosphamide (2/15), romiplostim (2/15), danazol (1/15) and splenectomy (1/15). Before eltrombopag treatment, the majority (8/15) showed grade 4 (WHO) thrombocytopenia. Initial dose was 50 mg and increased to 75 mg daily in 3/15 pts and in combination with corticosteroids that were gradually tapered by the 5th week in 12/15. Median platelets value by the 2nd week of administration was 140x10^9/L (54-450 x10^9/L); whereas, by the 4th week increased to 185x10^9/L (16-500x10^9/L). At the end of follow up, all patients but one achieved CR with median platelets of 145x10^9/L (60-400 x10^9/L). Regarding adverse events, 1/15 pt presented hemolytic anemia, 1/15 pt hepatotoxicity grade 2 with episodes of thrombocytopenia grade 4 and 1/15 pt pulmonary embolism during the second month of treatment. The latter 2 pts switched to romiplostim. Romiplostim was administered in 9 pts, 4 male:5 female with a median age of 66 years (33-76 yrs) for 6.7 months (3-11 mo). They had received a median of 3 previous treatments (range 1-8); corticosteroids (9/9), intravenous immunoglobulin (6/9), rituximab (6/9), vincristine (2/9), cyclophosphamide (2/9), eltrombopag (2/9), danazol (1/9) and splenectomy (2/9). The majority (5/9) presented thrombocytopenia grade 4 before romiplostim. Median platelets number by the 2nd week of administration was 50x10^9/L (8-140 x10^9/L); whereas, by the 4th week increased to 115x10^9/L (20-400x10^9/L). At the end of follow-up, 6/9 pts achieved CR with median platelets at 145x10^9/L (110-400x10^9/L). All patients received concomitant steroid treatment that was gradually tapered and stopped in 6/9 pts. 2/9 pts switched to eltrombopag due to thrombocytopenia grade 3 and 1/9 pt to danazol and low-dose steroids achieving CR. No adverse events associated with romiplostim treatment were reported. No significant differences were found between the 2 treatment groups. All 4 patients that switched to the other agonist achieved CR without adverse events.

Summary/Conclusions: Our real-world data suggest that both eltrombopag and romiplostim are safe, well tolerated and highly effective in refractory cITP and furthermore, switching to another agonist is safe and effective. Future studies will determine predisposing factors for adverse events and more accurate classification of patients that will allow for better treatment guidance.
D receptor (VDR) polymorphisms in the development of autoimmune diseases. Vitamin D affects both innate and adaptive immune responses that have been blamed in immune thrombocytopenia (ITP) pathogenesis.

Aims: The aim of this study is to 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 ($\chi^2 = 8.77, P = 0.001$). The BsmI polymorphism B allele was higher in ITP group than that in controls and it was statistically insignificant difference ($\chi^2 = 2.125, P = 0.145$). B 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 or 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 to 32 weeks, 36% starting 1g/kg with 1g/kg for 20 weeks. 23% referred to feto-maternal specialist to decide IVIG. Just 20% give 0.5mg/kg of steroids from 20 or 32 weeks. For high risk pregnancies defined as confirmed antibody positive with previous intracranial haemorrhage (ICH) after 28 weeks: 36% of centres used IVIG 1g/kg from 20 weeks, 36% give 1g/kg from 20 weeks increasing to 2g/kg at 32 weeks with 14% giving 2g/kg from 20 weeks and 14% initiating at 12 weeks. 40% gave 0.5mg/kg of steroids from 12-32 weeks starting. 60% of centres use a very high risk protocol (ICH before 28 weeks) with more intensive IVIG starting at 12 or 20 weeks with steroids of variable intensity and duration. Finally respondents were questioned whether there was a planned delivery time and method for the pregnancy? 58% plan a delivery at 38 weeks with no specific delivery mode. 18% plan delivery at 38 weeks by caesarean section, 8% plan a caesarean section but with no set time and 16% have no specific protocol plan for delivery.

Summary/Conclusions: The results of this survey reveal that the optimal medical management for the prevention of NAIT, namely the medication, dosage and schedule vary widely reflecting the lack of good evidence to guide centres in this very challenging area. Based on this survey we plan with our colleagues in UKOS a prospective study of treatment and outcomes.

PB2106
IMMUNE THROMBOCYTOPENIA AND PREGNANCY: A SPANISH CASE SERIES OF 270 PREGNANCIES IN PRIMARY ITP
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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. 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, lead to a supplementary increase in ROS levels and increased platelet destruction.

Aims: To evaluate management and results of pregnancy and delivery on pregnant ITP women and on their offspring.

Methods: All women diagnosed of primary ITP (according to international consensus criteria) from 2011 to 2016 in 23 Spanish Hematology Departments who had at least one pregnancy after ITP onset were included in this registry.

Results: We included 270 primary ITP pregnancies from 184 women. At pregnancy diagnosis, we observed a majority of chronic ITP cases (71.4%). At ITP diagnosis, median age of our case-series was 23 years (IQR, 19-29) and median time from ITP diagnosis to pregnancy was 167 months (IQR, 0-366). Median number of pregnancies prior to ITP diagnosis were 1 (IQR, 0-2) with 1 pregnancy (IQR, 1-2) after ITP diagnosis as a median.

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50.8% of women received corticosteroids, immunoglobulins (IVIG) (16.9%), rituximab (6.8%) and splenectomy (8.4%). All were on ITP treatments between or before new pregnancies. On the other hand, 26.4% of women needed treatment for ITP during pregnancy, mainly steroids (13.5%) and IVIG (10.2%). The median platelet-count nadir during pregnancy was 74 x 10^9/l (IQR, 36-172). 127 (47%) pregnancies suffered from non-haemostatic platelet levels (less than 50 x 10^9/l) with 73 (27.0%) women who achieved less than 30 x 10^9/l. 56 (20.7%) women exhibited hemorrhagic symptoms, being 30 (11.1%) of them severe bleedings. Regarding type of delivery, this was vaginal in 63.4% of pregnancies and cesarean sections 30.5%. Median platelet count at delivery was 110 x 10^9/l (IQR, 72-181). 43 patients (23.4%) experienced 57 bleeding episodes. We only observed 48 cases (20.4%) of neonatal thrombocytopenia among 235 living newborns.

Summary/Conclusions: Our results are comparable to previously reported studies. No severe bleeding complications during pregnancy and/or delivery were observed in our case series. Rate of neonatal thrombocytopenia, and therefore, newborn bleeding is low.

PB2107
ANALYSIS OF THE DEMOGRAPHIC, CLINICAL, LABORATORY AND TREATMENT-RELATED DATA OF ITP PATIENTS IN GREECE BASED ON THE NATIONAL ITP REGISTRY OF THE HELLENIC SOCIETY OF HAEMATOLOGY

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Background: Immune thrombocytopenia (ITP) consists of various acquired disorders caused by autoantibodies against platelets resulting in increased platelet destruction and impaired thrombopoiesis. ITP is characterized as primary when an underling etiology cannot be identified and secondary when a causative etiology exists. Data concerning ITP characteristics at a national level are limited.

Aims: The purpose of the study was to access systematically the demographic, clinical, laboratory and treatment-related data of ITP in Greece based on the national database (ITP registry) operated and supported by the Hellenic Society of Haematology.

Methods: 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 and a wide variety of symptoms are seen on initial presentation.

Aims: A retrospective review of the significance of specific symptoms and their duration on mortality.

Methods: A retrospective review of all consecutive admissions to a single tertiary center between 2009 and 2015. Only patients who required plasma exchange were included. Patients’ symptoms and their duration were reviewed in addition to presenting anti-ADAMTS13 IgG antibody levels and ADAMTS13 antigen levels, both of which have previously been found to have prognostic significance.

Results: 106 patients (68% female) were included with a median age of 48. 58% were Caucasian and 19.8% Afro-Caribbean. The mortality rate was 7.4% (n=8). 47% of patients had neurological symptoms on presentation, 24% reported a bleeding history and 12% a recent infection. The most common presenting symptoms were headache (27.4%), bleeding (24%) spontaneous bruising/ petechiae (19.8%), and chest pain (19.4%). Patients with anti-ADAMTS13 IgG level was not however significantly higher in these symptoms when compared to others (Table 1) suggesting microangiopathic thrombosis location plays an important role in TTP prognosis. The median duration of symptoms prior to presentation was 7 days (range 1-60 days). 8.5% of patients were asymptomatic, 16.2% were diagnosed with TTP. Patients in the highest quartile for symptom duration (>10 days) had significantly higher anti-ADAMTS13 IgG antibody level than those in the lowest quartile for symptom duration (<2 days) (65% vs 26%, p<0.002) and may have increased mortality (symptoms <2 days mortality 7.4%, symptoms >10 days 14.3%, p=0.19).

Table 1.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Mortality (%)</th>
<th>Antibody (NR-40%)</th>
<th>Antibody (NR-70%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>21%</td>
<td>26%</td>
<td>50%</td>
</tr>
<tr>
<td>Headache</td>
<td>25%</td>
<td>44%</td>
<td>44%</td>
</tr>
<tr>
<td>Speech Disturbance</td>
<td>4%</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Thrombotic events</td>
<td>4%</td>
<td>8%</td>
<td>8%</td>
</tr>
<tr>
<td>Leukocyte (15)</td>
<td>32%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Spontaneous bruising (15)</td>
<td>35%</td>
<td>20%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Whilst there is little difference in the anti-ADAMTS13 IgG antibody and ADAMTS13 levels seen with different symptoms, there is a wide disparity in terms of mortality suggesting the effect of microangiopathic thrombosis differs by location. Abdominal pain, not previously recognized as a key finding in the diagnosis of TTP, may be a poor prognostic marker. Although this should be interpreted with caution given the sample size. Anti-ADAMTS13 IgG antibody level increases with symptom duration and this may lead to increased mortality.
PB2109
NOVEL TECHNIQUES FOR MONITORING 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 perioperative period. We herein describe our experience with monitoring the proportion of donor platelets following transfusion, and their contribution to whole blood clot formation.

Aims: To describe the use of flow cytometry (FC) analysis in order to detect donor transfused platelets in a GT patient undergoing a minor surgical procedure and to assess the correlation between FC analysis and the results of Rothenberg Thromboelastography.

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 platelets FC demonstrated 20.6±1% donor platelets equivalent to 55,620 donor platelets. Platelets activation was determined following ADP addition by examination CD62 antigen expression. Seven days post platelet transfusion FC demonstrated 2.6% equivalent to 8,658 donor plantlets. The decline in the number of active platelets was associated with a reduced clot firmness (MCF) and lower a-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 the useful for splenectomy indication.

Figure 1.

PB2110
CAN HISTOCHEMICAL C-MPL POSITIVITY IN BONE MARROW BE A PREDICTOR FOR SPLENECTOMY IN IMMUNE THROMBOCYTOPENIA?
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Background: Splenectomy is used as the second line therapy in patients with immune thrombocytopenia (ITP). However, there is no parameter predicting splenectomy decision.

Aims: Aim of the present study was to evaluate immune histochemical Cloned Myeloid Leukemia Virus (c-mpl) positivity in bone marrow specimens of ITP patients with or without splenectomy indications.

Methods: Bone marrow specimens were taken from 24 patients who were diagnosed with ITP and who had splenectomy (15 female, 9 male, mean age 50±16) before splenectomy and 30 patients who were diagnosed with ITP but did not have splenectomy (15 female, 15 male, mean age 52±19). c-mpl staining was carried out retrospectively. Immuno-histochemical (IHC) staining using Avidin-Biotin complex system (ABC) was conducted. For IHC, sections prepared from blocks were taken onto poly-L-lysine coated slides (MicroSlides Snowcoat X-tra). Dissections were treated with IHC-c-mpl (Santa Cruz sc-13187) stain. Cytoplasmic and nuclear staining was observed in megakaryocytes using IHC c-MPL and vitamin D. Evaluation was made based on the intensity of staining, i.e. negative (0), weak (1+), moderate (2+) and strong (3+) (1). All samples who had splenectomy were in chronic phase of the disease. The present study was supported as a Scientific Research Project by Adnan Menderes University (TFP-15027).

Results: c-mpl positivity was statistically significant in patient group who did not have splenectomy (Table 1). In patient group who had splenectomy, c-mpl was not associated with refractory status.

Table 1. c-mpl positivity in patient groups who had and did not have splenectomy.

Summary/Conclusions: Status of c-mpl in ITP is ambiguous. Significant level of positivity in patient group who did not have splenectomy might be a useful for splenectomy indication.
Summary/Conclusions: Thrombocytopenia is a potential risk of bleeding during the labor. A high IPF indicates either consumptive or recovering thrombocytopenic disorders, such as immune thrombocytopenic purpura, while low IPF is characteristic of bone marrow suppression states. Although not directly used in clinical decision making, the reference range is critical to the introduction of new parameters and the interpretation of laboratory results. Our results suggest that the normal limits of this parameter are determined, and a level <10% might be an independent bleeding factor which can be useful for detecting high-risk pregnant patients. It should be corroborated in further studies.

Background: Immune thrombocytopenia (ITP) is an acquired, immune-mediated disease that is characterized by increased destruction of platelets by autoantibodies. ITP is characterized by mucocutaneous bleeding. Rarely, life-threatening bleeding such as central nervous system bleeding can occur. Typically, patients have isolated thrombocytopenia. The diagnosis of ITP is one of exclusion. Corticosteroids are chosen as a first-line therapy for adult patients who require treatment. Responses to first-line therapy with corticosteroids is about 80% with approximately 20% to 30% long term complete remission. Most patients finally relapse, requiring second-line therapy.

Aims: Our aim was to evaluate the relationship between the 3rd and 7th day platelet counts as predictors of early platelet response to corticosteroid therapy on achieving long-term complete remission.

Methods: We retrospectively evaluated 43 ITP patients who were followed-up at our institution. All patients' platelet counts were below 30 x10^9/L at diagnosis. All patients received initially methylprednisolone (MP) 1 mg/kg/day. For patients who responded with platelet count ≥150 x10^9/L methylprednisolone was tapered over 3 months. Those who were unresponsive to MP or relapsed after a complete response, were treated with second-line therapies that splenectomy or medical treatment agents. The platelet counts of the patients after a complete response, were treated with second-line therapies that included corticosteroid therapy on achieving long-term complete remission.

Results: Baseline characteristics of the cohort of 43 patients with an initial diagnosis of ITP are shown in Table 1. The mean age at diagnosis was 51 years (18-84) with female/male = 25/18. All patients presented with severe thrombocytopenia (platelet counts below 30.0 x10^9/L). Most patients presented with mucocutaneous bleeding (n=39), only three patients had genitourinary or gastrointestinal bleeding and one patient was asymptomatic. Bone marrow aspiration and biopsy was done in 14 (32.6%) patients due to various reasons including persistent bleeding. Reduced peripheral smears examination. Effect of the platelet counts on day 3 and 7 for each patient were compared, a significant association was found in correlation analysis (p=0.04). When platelet counts on the 3rd and 7th day in each patient were compared, a significant association was found in correlation analysis (p<0.05).

Summary/Conclusions: For patients who responded with platelet count ≥150 x10^9/L methylprednisolone was tapered over 3 months. Those who were unresponsive to MP or relapsed after a complete response, were treated with second-line therapies that included corticosteroid therapy on achieving long-term complete remission.

Background: Chronic primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by enhanced clearance of platelets and impaired platelet production. Corticosteroid is the ministry line of treatment of ITP, patients who fail to respond 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 monoclonal antibody (mAbs) for mechanism of action of rituximab, it targets the antibody-dependent cellular cytotoxicity (ADCC), ADCC effectiveness is influenced by process of activation of effector cells via their immunoglobulin G fragment C receptors (FcRγ). Fcγ receptors show distinct affinity to bind to IgG subtype specificities. Differential response to rituximab has been reported to correlate with specific polymorphisms in two of Fcγ receptors, FcγRIIa (H131R) and FcγRIIIa (V158F) in some diseases.

Aims: To clarify the effect of FcγRIIa-131 R/H and FcγRIIIa-158 V/F genes polymorphism on the response to rituximab in ITP patients.

Methods: We studied the frequency of the FcγRIIa (H131R) and FcγRIIIa (V158F) gene polymorphism in 100 chronic ITP patients; 50 patients (44.4%) carried FcγRIIa RR genotype and 7/18 patients (38.9%) carried FcγRIIa HR genotype. By the end of month 3 of the second line therapy, 13 patients (26%) achieved NR. Among the 50 patients who treated with Rituximab; 18 patients (36%) achieved CR, 19 patients (38%) achieved PR and 13 patients (26%) achieved NR. Out of the 18 patients who achieved CR, 8/18 patients (44.4%) carried FcγRIIa RR genotype and 7/18 patients (38.9%) carried FcγRIIa HR genotype. However it was not statistically significant. Among the 13 patients who achieved NR; lowest rate was patients carried FcγRIIa RR genotype (23.1%) compared to HR (38.5%) and V/F (38.5%) genotypes. However it is not statistically 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 who 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).

PB2114 IMMUNE THROMBOCYTOPENIA, EGYPTIAN EXPERIENCE WITH STUDY OF IL-17, TGFβ-3, IL-35 AND IL-12 CYTOKINES IN CHRONIC AND PERSISTENT IMMUNE THROMBOCYTOPENIA PATIENTS

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Background: The role of T cells in the pathophysiology of immune thrombocytopenia (ITP) is heterogeneous and complex. It has been studied in active and reactive ITP but not to same extend in chronic and persistent type.

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-3 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 which all cases were positively correlated to each other in addition to another immunesuppression 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 lower expression of IL-35 and IL-12 might be due to persistent production of cytokines due to expression of cytokines production by effect of immune suppression use or up regulation of their receptors on Treg cells which have resistance to their activity. In chronic ITP, the level of T cell cytokines can't predict the course of disease.
PB2115

SWITCH OF TPO-MIMICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FLORENCE MONOCENTRIC EXPERIENCE

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Background: Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count <100,000/μl in the absence of an identifiable underlying cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that throbopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction.

Aims: We evaluated patients treated with both TPO-mimetics.

Methods: From November 2008 and February 2017, 65 patients were treated with TPO-mimetics with a median follow-up of 29 months (1-96); 39 patients underwent therapy with Romiplostim and 26 to Eltrombopag. In our study we evaluated 18 patients who received both therapies: among patients treated at first with Romiplostim, 10 patients (9F; 1 M) switched to Eltrombopag and 8 patients (3 M; 5 F) switched from Eltrombopag to Romiplostim. In the group of 10 patients treated at first with Romiplostim, 5 patients started Eltrombopag because were no responders, 3 for loss of response and 2 patients because of adverse events. In the group of 8 patients at first treated with Eltrombopag, 4 patients didn’t obtain any response with Eltrombopag and switched to Romiplostim, 1 patient underwent to Romiplostim for loss of response and 3 patients because of adverse events.

Results: Among patients switched from Romiplostim to Eltrombopag, 2 achieved complete response, 4 response and 4 were no responders; among patients switched from Eltrombopag to Romiplostim, 4 obtained complete response, 3 response, 1 no responder.

Summary/Conclusions: Romiplostim and Eltrombopag stimulate the TPO-R but have different mechanisms of action, therefore, in our limited experience switching from one thrombopoietic receptoragonist to the other could be beneficial in clinical practice for patients with severe chronic immune thrombocytopenia who failed to respond or experienced adverse events to the first treatment.

PB2116

COEXISTENCE OF GLANZMANN’S THROMBASTHENIA AND MAPLE SYRUP URINE DISEASE: IMPLICATIONS FOR HEMOSTATIC MANAGEMENT

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Background: In Oman, autosomal recessive disorders are relatively commoner than western communities due to the high prevalence of inter-tribal marriage. Unfortunately, some patients have got more than one autosomal recessive genetic disorder, owing to complex consanguinity which might further complicate proper management plans.

Aims: To report an interesting case of combined Glanzmann’s thrombasthenia and MSUD.

Methods: Case report and retrospective data analysis of all cases with confirmed or suspected platelet function disorders in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2006 till December 2016.

Results: In a 4 year-old girl who is a known case of MSUD. Her parents are double first cousins (from both maternal and paternal sides). At the age of 3 months, she required Gastrostomy tube (G-tube) insertion. Preoperatively, she required multiple blood products, but bleeding continued. The patient had massive hematemesis. The patient received multiple blood products, but bleeding did not stop. As an emergency measure, recombinant activated factor VII (rFVIIa) was given and resulted in cessation of bleeding. Platelet aggregation studies revealed defective aggregation with ADP, arachidonic acid, collagen and epinephrine which is consistent with Glanzmann’s thrombasthenia. The diagnosis was further confirmed by platelet aggregation which showed no activity with CD41 and CD61, indicating absent GpIIb/IIIa complex. The patient experienced a severe bleeding phenotype, which is further complicated by multiple coexisting factors, including the recurrent episodes of metabolic crises which provoked worsening of platelet function, the development of platelet refractoriness at the age of 1 year, and the need for recurrent invasive procedures such as G-tube and central line insertion. Currently, the bleeding episodes are managed by rFVIIa at a dose of 120-180 µg /kg/dose. Excluding von Willebrand disease, we have 38 cases of confirmed or suspected platelet function disorders in our center, including 15 cases with Glanzmann’s thrombasthenia, 7 cases with Bernard-Soulier syndrome, 5 cases with May–Hegglin anomaly and 11 cases of suspected, yet unconfirmed platelet storage pool deficiency.

Summary/Conclusions: In conclusion, children with platelet function disorders still have plenty of unmet needs, ranging from deficient accurate diagnostic facilitation that can lead to inappropriate management guidelines. The coexistence of another hereditary disorder may result in mutual management difficulties of both diseases. In developing countries, proper registry is needed to establish optimum care of such rare disorders.

PB2117

ASSESSMENT OF PLATELET REACTIVITY TO ASPRIN AND CLOPIDOGREL WITH POINT-OF-CARE VERIFYNOW® 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-aggregant effect of aspirin and, specially, with clopidogrel has been reported. The VerifyNow® System (Accutrendms, 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 response 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 <47 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 thienopyridines, we performed it to analyze whether 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® 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.

<table>
<thead>
<tr>
<th>Platelet Function Test</th>
<th>VerifyNow®</th>
<th>Multiplate®</th>
<th>PFA-100</th>
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<tbody>
<tr>
<td>Aspirin sensitivity</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PRU &lt;85</td>
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<tr>
<td>P2Y12 U &lt;200</td>
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<tr>
<td>ADP aggregation &lt;47 U</td>
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<tr>
<td>AA aggregation &lt;47 U</td>
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</tr>
<tr>
<td>Clopidogrel sensitivity</td>
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</tr>
<tr>
<td>PRU &lt;85</td>
<td></td>
<td></td>
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<tr>
<td>Collagen aggregation &lt;47 U</td>
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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
ADP is not good enough to detect clopidogrel-mediated platelet dysfunction since it is not specific for the P2Y12 receptor. The addition of PRF to the ADP test may increase its sensitivity. VerifyNow® assay seems to underestimate the effect of clopidogrel, since hyper-response data are not reproduced by other techniques. According to our results, a high interindividual variability in response to clopidogrel is observed.

**PB2118**

**THROMBOPOIETIN-RECEPTOR AGONISTS IN ITP - EXPERIENCE OF A CENTER**

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**Background:** Thrombopoietin-receptor agonists (TRA), romiplostim and eltrombopag, are part of the treatment of chronic immune thrombocytopenia (ITP), resistant to first line therapy (corticosteroids and/or immunoglobulins) and with a significant bleeding risk. Both are approved for 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 in response to TRA, clinical evolution and adverse events 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 (86.8%) patients were treated with eltrombopag (5 pediatric cases): 27 patients (81.8%) responded while 6 patients had resistant disease (3 of these were HIV positive). The response rate was higher in patients with previous splenectomy (91.7% with romiplostim and 92.9% with eltrombopag) compared to those with no previous splenectomy (25% with romiplostim and 73.7% with eltrombopag). Six patients maintained response after treatment suspension (5 treated with eltrombopag and 1 treated with romiplostim). Generally, both treatments were well tolerated, with only one case of eltrombopag suspension because of a thromboembolic event.

**Summary/Conclusions:** In the current study, both TRA were effective in the treatment of ITP resistant to several lines of treatment, with similar response rates.

An important part of our current work is to define the optimal period of treatment and surveillance, especially in pediatric patients. In our center, the median time of treatment with eltrombopag for all patients was 5.5 months (range between 1 to 34 months) and with romiplostim was 12 months (range between 1.5 to 85 months). The duration of treatment with eltrombopag in children and adolescents was around 6 months.

**PB2119**

**THE EVALUATION OF REACTIVE OXYGEN SPECIES IN ESSENTIAL THROMBOCYTOSIS AND CORRELATION WITH JAK2V617F MUTATION**

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**Background:** Essential thrombocythemia (ET) is a clonal disorder of the hematopoietic stem cells characterized by excessive myeloid proliferation, with predominant megakaryocytic expansion and a potential transformation to acute myeloid leukemia. 50 to 60% of ET cases present a JAK2V617F mutation. 5% to 10% of JAK2V617F-negative ET patients have MPL mutations at codon 515 and 50% to 70% of ET patients with non-mutated JAK2 and MPL (double-negative) mutation are co-expressing of CALR. Genetic instability in ET patients is associated with an increased level of reactive oxygen species (ROS) which also leads to genomic instability and transformation to acute myeloid leukemia.

**Methods:** We studied 23 patients with ET admitted to the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, diagnosed with ET according to the 2008 revised WHO criteria (informed consent obtained). All analysis were performed after diagnosis and before the start of therapy. The JAK2V617F mutation was detected by allele specific polymerase chain reaction (PCR) testing. ROS levels were detected by flow-cytometry using a Cy Flow Space Systex flow-cytometer and a DCFDA Cellular ROS Detection Assay Kit. Studied parameters were compared both to healthy controls and to each other. Exclusion criteria were patients with any condition associated with an increased oxidative status (alcohol consumption, smoking, diabetes mellitus, hyperlipidemia, chronic renal failure, human immunodeficiency, cirrhosis, and active infection), use of antioxidants or iron supplementation. Data analysis was performed using Flow Max software. The differences between the two groups were assessed using the Student T-test and a p-value of less than 0.05 was considered statistically significant.

**Results:** The study group involved 12 females and 11 males, with a median age of 48 years. All patients had increased ROS levels at diagnosis compared to healthy controls. Eleven patients had JAK2V617F mutation and twelve were JAK2V617F mutation negative. Significantly higher ROS levels were found in JAK2V617F negative patients compared to JAK2V617F positive 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.

**PB2120**

**VARIATIONS IN PARAMETERS OF PLATELET COUNT AND PLATELET VOLUME ACCORDING TO GESTATIONAL AGE**

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**Background:** Reference ranges of haematological parameters in preterm infants are limited.

In hematological evaluation not only platelet (PLT) counts but also 3 important platelet volume parameters (mean platelet volume [MPV], platelet distribution width [PDW], plateletcrit [PCIT]) are also taken into consideration.

**Aims:** We wanted to investigate the impact of gestational age by determining variations in platelet volume parameters according to gestational weeks.

**Methods:** Medical records were prospectively reviewed in preterm infants admitted to Firat University Hospital from January 2001 to December 2007. Study group consisted of only one-hour-old newborns delivered in the clinics of Department of Gynecology, and Obstetrics of our hospital. The exclusion criteria included those with maternal history of antepartum haemorrhage, chorioamnionitis, fever, sepsis, preeclampsia and hypertension; and perinatal history of twin-to-twin transfusion syndrome, feto-maternal transfusion, infection and infection. A hundred and ninety-three newborns with apparent health problems were excluded from our study. Study group comprised 398 preterm infants born between 26-37 gestational weeks, and 63 healthy term (38 gestational weeks) infants. MPV and PDW from all cases were obtained within the first hours after birth. Blood samples were placed into tubes with EDTA, and analyzed in ADVIA 120® (Japan) hematology analyzer using suitable kits. Data were expressed as mean±standard deviation. Platelet counts, and volume were indicated for each gestational week, and groups of 24-31, 32-36, 37, and 38 weeks. One-way analysis of variance (ANOVA) was used for statistical analysis, and p<0.05 was accepted as the level of statistical significance.

We established the reference ranges of platelet and platelet index in Turkish preterm and term infants. Platelet counts, and platelet volumes continually change as gestational age increases. Increases in platelet counts, and PCT, while decreases in MPV and PDW were detected. The gestational age-related changes in PLT patterns may reflect maturation of platelet regulation.

**Results:** Platelet counts increased beginning from the 26th up to 28th weeks. They did not change between 29th and 33rd weeks, while their levels risen again consecutively between 34th and 37th weeks. At 38th week a dramatic increase of platelet counts occurred (P<0.05). MPV and PDW from all cases were obtained within the first hours after birth. Blood samples were placed into tubes with EDTA, and analyzed in ADVIA 120® (Japan) hematology analyzer using suitable kits. Data were expressed as mean±standard deviation. Platelet counts, and volume were indicated for each gestational week, and groups of 24-31, 32-36, 37, and 38 weeks. One-way analysis of variance (ANOVA) was used for statistical analysis, and p<0.05 was accepted as the level of statistical significance.

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**PB2121**

**RISK OF LUPUS AFTER PRIMARY IMMUNE THROMBOCYTOPENIC PURPURA: A 14 YEAR SINGLE CENTER EXPERIENCE**

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease in which antibodies develop against platelets (plts) and dysregulation of cellular immunity result in premature destruction of plts and impaired plt production. For most affected children, ITP is a self-limiting disease. Approximately, 10% of 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.

Methods: All patients diagnosed with ITP and with a platelet count <100x10⁹/L, as well as ANA positive patients with initial positive ANA are at risk for development SLE. Therefore, we included patients diagnosed with ITP and with a platelet count <100x10⁹/L.

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

INVESTIGATION OF PLATELET FUNCTION 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-aseticacid (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. This situation 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 occurs.

Aims: In theory, formation of platelet clusters in the presence of EDTA requires 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 x10³/µL) and value of this new method for determining pseuTCP and real-TCP. There was no study investigated this system for this purpose. Descriptive analyses were presented using means zstandart 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 preliminary results.

Results: We included 59 patients who were referred to our clinic with thrombocytopenia (TCP, Plt<150 x10³/µL) 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 x10³/µL (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-TCP and real-TCP. There was no difference in terms of Plt, MPV, PCT, WBC or HB between these groups but age was younger variant (median age 46 vs 62, p<0.05) and PDW was lower 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<0.005 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 sensitivity and specificity were 74.4% and 95%, with overall accuracy of 81.4% for ColEPI (AUC 0.813, %95CI: 0.689-0.933). 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 thrombocytopenic cases, could be used for differential diagnosis between 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, Timișoara.

Methods: A retrospective study for 350 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient’s medical charts for the 15 months prior to their most recent visit.

Results: The mean age was 45.6 years with 58% women and 42% men. Median time from the diagnosis of ITP to the start of the observational period was 23 months. Regardless of the presence of bleeding symptoms, for majority of patients we started treatment based on platelet count. Treatment was considered when platelet counts are less than 20x10^9/L in patients without bleeding, and less than 30x10^9/L in patients with bleeding. Prior to the observational period, 36% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 72% of all patients were treated. The most frequent reasons given for treatment were platelet count (58%), followed by bleeding symptoms (42%). Corticosteroids represented 52% of treatments, followed by IVIg (20%), azathioprine (12%) rituximab and 8% others. Splenectomies (8% of patients) and platelet transfusions (27% of patients) were performed during the observational period. In the patient survey, 52% of participants were 60 years of age or older and the duration of disease was more than 10 years in 43% of patients. The minimum platelet counts were less than 10x10^9/L in 49% of patients. The most common symptoms of ITP was fatigue (45%). Approximately 60% of patients reported at least one side effect associated with ITP treatment. The side effects were most frequently associated with corticosteroid use (43%). Overall, 40% of patients required hospitalization. Mean duration of hospitalization was 13.5 days.

Summary/Conclusions: The retrospective study of 350 patients provides the results of treatment practices in our country. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids were the most widely used treatment.

PB2125

IMMUNOLOGICAL THROMBOCYTOPENIC PURPURA AND PREGNANCY: A RETROSPECTIVE STUDY OF 89 PREGNANCIES IN 59 PATIENTS

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Background: Immunological thrombocytopenic purpura (ITP) occurs for about 1 case for 1000 pregnancies. The risk of onset, aggravation or relapse of ITP during pregnancy is not clearly established.

Aims: The aim is to describe the prevailing ITP progression profile in pregnant women and to evaluate the risk of neonatal thrombocytopenia in two situations, when ITP was known before pregnancy and when ITP was discovered for first time during pregnancy.

Methods: It is a retrospective study carried out in the hematology department of CAC Blida, Algeria, between 1993 and 2016. All patients (pts) who had a pre-pregnancy ITP or thrombocytopenia during pregnancy attached to an ITP were included.

Results: The development of 89 pregnancies (PG), including two twins, occurred in 59 women was analyzed. There were one PG in 40 pts, 2 PG: 13 pts, 3 PG : 5 cases, 4 PG : 1 case and 5 PG : 1 case. Of the 59 pts: in 42 cases it was a history of ITP before pregnancy (group 1: G1) with a history of splenectomy in 9 patients, and in 17 cases it was ITP discovered on the occasion of Pregnancy (group 2: G2). The average age at diagnosis was 26.7 years (7-44) and that at delivery was 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, abdominal 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 eventual to be evoked in front of a thrombocytopenia of the pregnant woman after elimination of the other causes related to the pregnancy and in front of the non-resolution after the delivery. The pre-existing ITP does not necessarily.
Quality of life, palliative care, ethics and health economics

PB2126
QUALITY OF LIFE AND SYMPTOM BURDEN IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Multiple myeloma (MM), the second most common hematological cancer, remains incurable. Its incidence is rising due to population ageing. Despite the impact of the disease and its treatment, not much is known about health-related quality of life (QoL) of patients with MM.

Aims: This study aimed to (1) Determine symptom prevalence in patients with MM on disease-modifying treatment, and identify the range and nature of these symptoms within the dimensions of physical, psychological, social well-being. (2) Measure the QoL of patients. (3) Compare the above-mentioned parameters to the general population.

Methods: Adults with multiple myeloma attending the hematology day unit in hematology department from November 2016 to January 2017 were eligible for inclusion in a cross-sectional. Consenting patients completed 2 validated questionnaires: 1) the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) 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 11.0). The QoL scores were significantly lower than the general population (54.7 vs 71.2). The most commonly reported physical symptoms were pain (72%), fatigue (70%) and insomnia (66%). About 61% of the patients were burdened by financial worries. On multivariate analysis, a good performance status (PS) 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 signiﬁcantly decreasing hemoglobin concentration and occur symptoms as fatigue, dizziness, palpitations, dyspnea markedly reduce patient activity, resulting in impaired Quality of Life (QoL).

Aims: To compare of QoL in HM’s patients with different grades of anemia.

Methods: In this study were included following patients (n=326) in the age of 19-82 (Me=65) years: myelodysplastic syndrome (n=37), acute myeloid leukemia (n=20), acute lymphoid leukemia (n=7), primary myelofibrosis (n=23), chronic myeloid leukemia in blast crisis (n=6), multiple myeloma in II and III st. (n=129), Non-Hodgkin’s lymphoma in III-IV st. (n=40) and chronic lymphocytic leukemia in B or C st. (n=67). Patients were examined: 1) clinical blood test (hemoglobin concentration) to assess anemia’s grade; 2) the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale to measure of QoL. The FACT-An questionnaire consists of a general questionnaire (FACT-G), measuring domains of physical well-being (PW), 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). Patients were divided into six groups according to the Hb concentration: 1) the first group – Hb was 4.0-6.4 g/dl; 2) the second – Hb 6.5-7.9 g/dl; 3) the third – Hb 8.0-9.4 g/dl; 4) the forth – Hb 9.5-10.9 g/dl; 5) the fifth – Hb 11.0-11.9 g/dl; 6) the sixth – Hb 12.0-14.4 g/dl.

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 S/FW – 14.2±0.7, in EW – 18.5±0.8, in FW – 27.8±1.3, in AnS – 34.7±1.6, in FS – 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 sells transfusions and erythropoiesis-stimulating agents can improve QoL in a clinically meaningful way.

AN ANALYSIS OF THE IMPACT OF LOCAL COSTS OF MEDICINES ON COST EFFECTIVENESS OF THE TREATMENT OF CANCER ASSOCIATED THROMBOSIS.
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Background: New research has surfaced in relation to health care resource utilization and costs in Cancer Associated Thrombosis (CAT). The studies originate from the US and are difficult to transfer directly to other countries. A few studies in Europe focusing on the total cost of CAT seem to indicate that the cost data in the field of CAT varies greatly between regions. To examine the importance of region specific cost elements in relation to research related to CAT, we studied the cost driver in the newest and most relevant health economic research and compared it with the costs from 6 European countries as well as Canada.

Aims: To highlight the importance of localized or regionalized cost inputs as cost drivers when considering cost effectiveness in relation to CAT.

Methods: The cost driver is the medication in a recent analysis by Connell 2016 and thus the focus of our analysis. The American paper incorporates outcomes from 6 RCTs for treatment with LMWH in patients with CAT. The annual medication costs of LMWH for daily treatment in 365 days were 32,120 USD in wholesaler acquisition cost (WAC). For VKA the annual medication cost for 365 days was 44 USD. LMWH is the cost driver but is not cost effective due to the cost of it. The study finds that the one-way sensitivity analysis shows that LMWH would become the preferred strategy once its annual cost was less than $7177. In the present analysis, the daily cost acquisition cost wholesaler Purchasing Price (WPP) (which corresponds to the American WAC ) for a LMWH (prefilled treatment kit) in the Netherlands with Tinzaparin was gathered in 7 large markets using a data retrieval from IHS global insights systems (Jan 2016). In addition to this, the role of the cost driver was also compared to other publications.

Results: Simply by applying the local unit cost for the treatment with LMWH for these countries, the conclusion becomes notably different. LMWH becomes the cost effective alternative in the European countries as well as in Canada with annual costs below 7177 USD. The price for VKA is comparable to that in the US, and does not change the cost effectiveness ratio. The data from the retrospective cost of CAT study that the cost of the hospitalization of 19% was 19% of the total cost of CAT and the CAT medication 11% of the total cost of CAT. This outlines hospitalization is a cost driver as well and not only the medication. Similar conclusions were reached in other studies. In summary, the role of the cost driver can change as a consequence of the localization of the costs. This outlines the great variation in costs in terms of CAT, and the caution it must be used with (Table 1).

Table 1.
MINIMIZING THE RISK OF MUCOSITIS IN HEMATOLOGIC PATIENTS WITH TOPICAL PRODUCTS
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Background: Mucositis is a frequent severe complication associated to aggressive therapies of hematological malignancies with chemos and/or radiation (therapy), conditioning therapy in stem cell transplants. Regularly occurs at 3 to 10 days after chemotherapy and about 6 to 8 weeks after radiotherapy. It is self-limited within 2-4 weeks, but in this period the patient is vulnerable to systemic infections (bacterial and fungal). It could also compromise the optimal timing and dosage of the chemotherapy schedule, induce psychosocial distress, prolonged hospitalization and finally, higher costs.

Aims: Evaluating the efficacy of GelX® in chemotherapy induced mucositis. GelX® is a topical product that contains Zinc gluconate+taurine, with bacteriostatic and anti-inflammatory effect, easy to use for the patient, in order to prevent and reduce pain and severity of oral ulcers, making a barrier for mucosae invasion. Methods: A retrospective analysis of 77 adult patients: 17 with hematological treatments and 60 with allologeneic stem cell transplantation. 17 were diagnosed and treated between January 2015 and December 2016 with various hematological malignances (5 AML, 2 ALL – 1 Ph positive, 2 blastic phases of CML, 3 AITL (CHOP/DA-EPOCH), 2 DLBCL (RCHOP), 1 FL (RCVP), 1 MM (radiotherapy), 1 Hodgkin disease (ABVD). Treatment regimens used for acute leukemias/blastic phases of CML were: ‘3+7’ (3 cases), MEC (1 case), high doses ARA-C (1), GMALL protocol (1), HyperCVAD (1), Idarubicine and ARA-C(1), HD-MTX(1). GelX® was indicated as prophylactic treatment for eight patients, because the risk of mucositis was high (aggressive chemotherapy, bad oral condition, risk of prolonged neutropenia). Curative treatment of grade 3-4 mucositis was indicated for 10 patients (one was initially treated with curative intention and after that with prophylactic treatment in) in 60 patients. The median duration of mucositis was 5 days varying from 0 to 8 days. There were 21 cases of sibling allotransplants (6 AML, 3 ALL, 1 ATLL, 5 LMML, 1 ALL C, 2 SAA, 2 CML, 1 mycosis) with 10 cases of mucositis grade 3-4. The regimens used were 6 mieloblastic and 15 nonmieloblastic. 3 from 4 cases of haplotransplant with nonmieloblastic conditioning (2MDs, 1 AML and 1 SAA) had grade 3 mucositis. Results: GelX® induced a reduction in the grading of mucositis (grad 1-2) and a shorter period of evolution (5 days) versus grade 3-4 mucositis and prolonged duration of oral lesions for those with curative treatment. From 60 patients allografted, 30 patients experienced grade 3 and 4 mucositis with a medium duration of five days. All of them received GelX® as prophylactic treatment.

Summary/Conclusions: Prophylaxis is the key of successful evolution in mucositis (time to heal shorter than 10 days). Identifying candidates for mucositis is mandatory and the product should be applied starting with the chemotherapy (or in the first 24 hours on the onset of chemotherapy) in order to minimize the risk of mucositis appearance.

EUROBLOODNET: THE EUROPEAN REFERENCE NETWORK IN RARE HEMATOLOGICAL DISEASES
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Background: Medical and patient organizations strongly recommend RHD. There was a need for a European reference network (ERN) on rare hematological diseases (RHDs) with a European reference network on rare and congenital anaemias (ENRCA), the European Haematology Association (EHA), and European hematologist 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. And EuroBloodNet’s main goal is to improve the healthcare and overall 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 rare diseases 4) Providing cross-border access to healthcare and research results 5) Furthering safe information, samples and patient mobility, b) provision of equal access to highly specialized procedures and innovative therapies resulting from best practice sharing, continuous medical education and virtual inter professional consultation for complex RHD cases, and c) facilitating of a timely and efficient trans-border research results into patient oriented strategy at the clinical and the public health level.

METHODS
RHDs are covered in two main thematic groups: non-malignant diseases (3) and sub-thematic areas. Non-malignant diseases include 4 sub-thematic areas: 1) Rare blood cell defects 2) Bone marrow failure (BMF) and hematopoietic disorders 3) Rare Bleeding-Coagulation disorders and related diseases and 4) Haemocromatosis 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.

Results: Expected outcomes include reduction of healthcare inequalities for RHD patients, by a) establishing a cross-border referral system allowing safe information, samples and patient mobility, b) provision of equal access to highly specialised procedures and innovative therapies resulting from best practice sharing, continuous medical education and virtual inter professional consultation for complex RHD cases, and c) facilitating of a timely and efficient trans-border research results into patient oriented strategy at the clinical and the public health level.

PB2130
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 are recommended to establish the diagnosis in those with the lower threshold (Arber DA et al,2016). This potentially could result in increased numbers and costs of investigations. The lower thresholds are aimed to identify those previously referred to as masked PV (mPV) who have been recognized to have an increased incidence of thrombosis (Barbui T et al, 2014 & 2015).We hypothesized that the revised criteria would increase the incidence of sub-clinical PV and potential PV who would then require additional investigations.

Aims: To determine number of patients with young strokes with potential PV on application of the 2016 revised WHO criteria for PV.

Methods: We undertook an analysis of records of patients with ischemic stroke from the Indo-US Stroke Registry and Infrastructure Development Project. This registry enrolled adult patients admitted with imaging-confirmed ischemic stroke <2 weeks after symptom onset. The Indo-US Stroke Registry and Infrastructure Development Project, includes 5 geographically diverse centres in India and one in USA. The registry data was entered into a well-based electronic database. From January, 2012 to March, 2017, 2076 patients with new onset ischemic stroke were evaluable in the Indian arm of the Indo-US Stroke Registry. We compared the incidence of polycythemia as determined that there was a statistically significant difference in the proportion of polycythemias, p = 0.000.

Results: There were 24 (1.2%) patients with potential PV which was revised to 60 (2.9%) patients with the 2016 Hb criterion. The McNemar test determined that there was a statistically significant difference in the proportion of polycythemias, p = 0.000. Considering the potential of comorbidities in the elderly to confound the association of polycythemia with ischemic stroke, we
separately analyzed only those with young stroke (Age <45). In this cohort there were 420 patients. A total of 6 (1.4%) patients had potential PV based on the 2008 Hb criteria. On applying the 2016 revision; 37 (8.8%) patients fulfilled the Hb criteria. An exact McNemar’s test determined that there was a statistically significant difference in the proportion of polycythemia, p= 0.000. Separate analyses by gender was not significant in females, P=0.5; but significant in males, p=0.000. More specifically, 57 (2.35%) males had PV. There were an additional 29 males with the revised criteria for polycythemia. The impact of cost in influencing treatment decision was resource limited countries with predominant out of pocket health expenditure has been earlier reported (Phillip C et al, 2015). This revision promotes the routine use of BM and JAK-2. In our analysis we estimate this new criterion would add to the costs to each patient (~ 7000 per patient estimate).

Summary/Conclusions: The present data shows that there exists a significant difference in the incidence of polycythemia in thrombosis (Ischaemic Stroke) on applying the revised criteria. The requirement to additionally investigate them with BM and molecular markers for PV has potential economic implications.

PB2132
PATHOPHYSIOLOGICAL MECHANISMS INVOLVED IN THE DEVELOPMENT OF ANEMIA IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA
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Background: Non-Hodgkin’s lymphomas (NHL) are a group of heterogeneous malignant lymphoid disorders that associate anemia either from diagnosis or during the evolution of the disease. The anemic syndrome can be present at the moment of diagnosis or can develop during the evolution of non-Hodgkin’s lymphomas, with clinical symptoms of anemia being a consequence of a decrease in the concentration of hematopoietic cells or due to reduction of extracellular volume. NHL can cause anemia due to decreased erythropoiesis and erythropoietin production, immunological mechanisms, bone marrow failure due to a metastatic process or due to a decrease in the concentration of erythropoietin. There are different mechanisms involved in the development of anemia in this study group. Various pathophysiologic 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 infiltration of malignant lymphomatous cells, cytopenias 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 pathophysiologic mechanisms involved in the development of anemia.

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 pathophysiologic mechanisms involved in the development of anemia in this study group. 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 involved and 26%. NHL replication on stage of disease was 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 pathophysiologic mechanisms involved in the development of anemia were: perturbations of iron metabolism and erythropoiesis under pro-inflammatory cytokines and hepcidin actions (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 origin was involved in 9.7% of cases. Five patients of anemia induced by chemotherapy and three patients with lymphomatous infiltration of the bone marrow also associated iron and/or folate deficiency. 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: 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 flulike or influenza-like illnesses or prior or concurrent diagnosis of autoimmune diseases were reported. Presenting symptoms may include focal neurologic deficits, neuroophthalmic symptoms, signs of increased intracranial pressure, seizures or ocular symptoms. Neuroophthalmic symptoms like depression, anxiety, psychosis, confusion, memory impairment, slowness of thought are generally undernoticed or underestimated due to the increased rates of depression and other cardiovascular manifestations. 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 antidepressants and anxiolytic drugs with their psychiatric diagnosis, time before the diagnosis of CNS lymphoma, the branch of the prescribing physician, presenting symptoms
from their medical files, type and treatment of lymphoma and survival were recorded. OECD international statistics as well as Turkish Statistical Institute data for national antidepressant use were collected and interpreted.

Results: Of the 40 patients, 14 were male (35%) while 26 were female (65%). Mean age was 60.5 years (38-78), 7 patients were alive (17.5%). Method for diagnosis was radiological imaging (magnetic resonance imaging) in 27 patients (67.5%) while in 13 patients, diagnosis was supported with histopathological confirmation (32.5%). Mean survival was 8.6 months (2-24 months). As the complaint for medical help seeking, 4 patients presented with neurophysiologic symptoms while 16 patients presented with headache (40%) and 20 patients (50%) presented with neurologic 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 onco-patients 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.

PB2135

IMPACT OF U.S. FDA APPROVAL OF LENALIDOMIDE MAINTENANCE THERAPY IN THE FIRST-LINE TREATMENT OF MULTIPLE MYELOMA AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION 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-auto-HSCT 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 revealed that the model results were robust to the variations of key model inputs.

Table 1.

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.

PB2136

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. A review of the medical charts 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 splenectomy 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 3000mL in SMH, and from 90 to 1500mL in SBH) were significantly higher in the SMH 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 SBH group was 10% and 13% for the SMH group (p=1). Late morbidity was 0% in the SBH group and 13% in the SMH group (p=0.26). 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 for SBH and SMH. For SMH, 14/31 patients received a pre-surgical corticosteroid treatment, with a pre-surgical platelets level of 156 +/- 108 vs 294 +/- 195 mL/109, white blood cell level of 15969 +/- 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 1013/mL, p<0.05). The median follow up is 39 +/- 37 months and 80% achieved a hematological recovery.

Summary/Conclusions: LS is a safe and less-invasive procedure in patients affected by Malignant Hemopathies. This approach is also well tolerated in older patients (median 67yrs) and in patients with large spleen (1515 +/- 660 mL), extending the indication for laparoscopic SHM even in older patient and in patients with high volume spleen. Compared to historical data, LSy for Malignant Hemopathies shows better early and late morbidities. Our data shows however a trend for higher late morbidity in the SMH group, warranting a careful long term follow-up in this subset of patients.

PB2137

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
patient due to urgent admission, long duration of stay in hospital, chemotherapeutic agents used in the treatment and the disease itself. Evaluating this group of patients for anxiety and depression, providing necessary professional support and revising medical treatment is therefore substantial.

Aims: In our study, we aimed to assess the risks of anxiety and depression in newly diagnosed acute leukemia patients who were admitted to hematology clinic to receive chemotherapy and provide necessary professional support along with treatment revisions and follow-up according to our findings.

Methods: Our study was performed with newly diagnosed acute leukemia patients, who were admitted to our hospital hematology clinic in a six-month period to receive chemotherapy. Demographic characteristics were noted and Hospital Anxiety and Depression Scale (HADS) was used to assess depression. Hospital Anxiety and Depression Scale (HADS) is an assessment scale developed by Zigmond and Snaith to determine the risks and assess the severity of anxiety and depression (8). The validation and reliability studies of the scale in Turkey were carried out by Aydiner et al (9). The questionnaire consists of 14 items: seven of which measure anxiety (odd numbers) and the remaining seven (even numbers) measure depression. Each item is scored from 0 to 3. The scoring order of each item in the questionnaire is different. Items numbered 1, 3, 5, 6, 8, 10, 11 and 13 indicate decreasing severity and are scored as 3-2-1-0. On the other hand, items numbered 2, 4, 7, 9, 12 and 14 indicate increasing severity and are scored as 0-1-2-3. The cut-off value for the total score of the odd-numbered questions assessing anxiety is 10; while it is 7 for the even-numbered questions assessing depression.

Results: 21 patients were included in the study. 13 of these patients (61.9%) were diagnosed with acute lymphocytic leukemia (ALL) and 8 (38.1%) were diagnosed with acute myeloid leukemia (AML). Median age of the patients was 45 (range: 21-69). 11 patients (52.4%) were female and 10 (47.6%) were male. 5 patients (23.8%) had comorbidities while 16 (76.2%) had none. Anxiety evaluation revealed that 38.1% of all patients in the study experienced anxiety. The rate of anxiety was 38.5% in ALL patients and similarly 37.5% in AML patients. 45.5% of the female patients had anxiety while the rate was only 30% in male patients. The difference was not statistically significant (p >0.05). Depression evaluation revealed that 81% of all patients in the study. The rate of depression was 84.6% in AML patients and 75% in ALL patients. 81.8% of the female patients had depression while it was 80% in male patients. Neither anxiety nor depression had a significant correlation with comorbidity or gender (p >0.05). Correlation analysis revealed a positive correlation between anxiety and depression (r=0.846; p <0.01).

Summary/Conclusions: In conclusion, assessing anxiety and depression in patients with acute leukemia is not only a crucial task in the course of and adherence to treatment. In our study, depression was distinctly more common than anxiety and there was a positive correlation between depression and anxiety. We think that including a professional for psychological support in the medical team is important for the treatment of these patients.

Background: Three years ago, a unit for autologous bone marrow transplant for hematological patients has been established in Shaare Zedek medical center. The patients meet with the doctors for the treatment plan usually following the diagnosis. From the point of view of a part of the patients, the process appears simple, short term, and promises cure. In reality, the process is long term, including aggressive chemotherapy prior to the transplant. The treatment is highly aggressive and toxic with many physical and mental side effects for the patient and her/his family. The transplant process requires hospital admission for about a month in an isolation room. No one is allowed in the room except for close relatives and the medical staff. The social worker, part of the caring staff, accompanies patients and families from the initial diagnosis through this taxing and stressful process. Most patients are young, average age 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 that bothered the patients during the phase of the preparation and the coping with it. The process of treatment helps patients to go from the private space 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.
Sickle cell disease

PB2140
HYDROXYUREA INHIBITS MYELOID DIFFERENTIATION VIA NITRIC OXIDE SYNTHASE
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Background: Hydroxyurea and nitric oxide (NO) inhibit erythroid differentiation, while hydroxyurea is NO-releasing agent used in therapy of sickle cell diseases in non-syndromic children have shown that sleep-disordered breathing (SDB) increases the risk of neuropsychological deficits and neuronal brain injury. Few authors have investigated the role in cognitive development 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 normalized NO dependence in inhibition of myeloid / erythroid differentiation, not influencing the hematological parameters.

PB2141
SLEEP DISORDERED BREATHING IN CHILDREN AND ADOLESCENT WITH SICKLE CELL DISEASE: IMPACT ON EXECUTIVE FUNCTION AND PROCESSING SPEED INDEX
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Background: Studies in non-syndromic children have shown that sleep-disordered breathing (SDB) increases the risk of neuropsychological deficits and neuronal brain injury. Few authors have investigated the role in cognitive deficits of SDB and the associated hypoxia in children with sickle cell disease (SCD). Snoring and SDB is very common in children with SCD and may affect cognitive function in very young children. Previous data suggested that executive function was worse in older children with SCD and low mean overnight oxygen saturation.

Aims: We aim to investigate if SDB could be a potential factor contributing to developmental problems in cognition in children and adolescent with SCD.

Methods: We have followed up children and adolescents in the Sleep Asthma cohort who underwent Polysomnography at two different time points (1) 2006-2009 and (2) 2011-2014 and compared the sleep data with subsequent neuropsychological assessment.

Results: Worse performance was found for processing speed: PSI (p<0.01) and general intelligence (p<0.05) compared to children without SDB. SDB, measured as apnea and hypoxia index (i.e. AHI >3%: Apnoeas and hypopnoeas with more than ≥3% desaturation), was found to impact executive function, as assessed with the Tower test. (p<0.05) and PSI (p<0.05). Mean oxygen saturation during total sleep time was significantly associated with lower PSI (p<0.05). Additionally, participants who showed a worsening of their SDB symptoms in their second sleep study had lower cognitive scores (i.e., executive function, p<0.05 and PSI, p<0.05) (Figure 1).

Summary/Conclusions: SDB symptoms seem to worsen into adolescence and therefore, might have a neurodevelopmental impact if left untreated; appropriate intervention might improve cognition and quality of life.
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 SCID 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 SCID patients included was 209. Ratio boy/girl is 1.3. Most of patients were born in Spain (85%), although 8% and 5.26% were born in Africa or America respectively. Seventy three percent of the progenitors came from Africa and 24% from America. Ninety two percent of those SCID 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/Betalath 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 throracic diathesis. Eighteen patients (8.65%) had human leucocyte antigen (HLA) iden-
tical siblings. Hydroxyurea was added to standard treatment in 65 patients (31%) of which 47 continue to be treated to date. Penicillin prophylaxis was communicated in 165 patients (79%). Vitamin-D prophylaxis was initiated in 122 patients (58%). Chronic exchange or present transplantsions existed in 25 cases (12%) and 9 children (4%) underwent splenectomy. None of these patients had sepsis or meningitis. Cholecyctectomy was performed in 9 cases (4%). There were 18 progenitor stem cell transplantations (8.61%) performed between 2.09 to 13.97 years of age (median 6.77 years). Ten patients remained on the waiting list, 4 were transplanted and 1 attained a marrow rejection. One patient died of graft-versus-host disease. Patients lost in follow up summed up 128: 23 for emigrating to other countries, 65 for continuing the monitor of their diseases in other centers or in adults units and 31 for unknown reasons. The number of patients was 84%.

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, a cell proliferation assay based on the susceptibility of a cell line to complement activation. Normal human serum (NHS) was used as a negative control and lipopolysaccharides (LPS)-incubated normal serum as a positive control. All samples were tested in triplicates and twice. Eculizumab containing serum (ECU) was added to the samples beforehand. A 96 well plate was used to test complement blockade 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.

Results: We studied 26 SCD patients (36±11 years of age, 12 male:14 female, 8 HbSS/S. 18 HbS/B-Thal). Among them, only 6 had a history of painful crisis and 15 a splenectomy, 15 were on hydroxyurea and 12 on antithrombotic treatment. Three patients had undergone previous studies and 8 patients had complement activation detected. Among these patients, 7 had a history of painful crisis and 6 of splenectomy. No significant differences were found regarding age, gender, platelets, white blood cells, Hb, Hbf, LDH and bilirubin levels between patients with and without complement activation. Then, we evaluated in vitro 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 activi-
tion 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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ology, University of Salerno, Salerno, Italy

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 imaging. 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 environ-

mental factors, like bilingualism or second language (L2) learning. The majority of children with SCD in Italy are of immigrant families whose first lan-

guage 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-demi-

graphically matched healthy controls recruited from elementary schools of the same school, performed an extensive battery of tests to assess naming skills, phonological and semantic fluency, attention and Executive Functions (inhibition and planning skills) and visuo-spatial skills (Boston naming test, phonological and semantic fluency tests, Flanker task, Eithorn test, PMA spatial relations subscale). All tests were administered in Italian. A composite index considering parental education and employment was used to match socially, demographi-

cally and economically children with SCD and healthy controls. An ad hoc battery of tests was developed to investigate the role of executive functions in SCD and healthy controls. Hierarchical regression analysis was 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 lan-

guage 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 chil-
dren 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 Vissuo-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 to 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

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was a factor of the lower performance in this task. Figure 1. Children’s Performance (in Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests. P-values: Visuo-spatial intelligence: not significant(n.s); Boston naming: ns; Phonol-Fluency: 0.004; Semantic fluency: ns.

at home to manage the onset of an acute crisis and the top 5 home strategies reported were: prescription pain medication (15%), sleep/rest (15%), apply heat using heating pad/blanket/bath/shower (13%), increase fluid intake (12%), and finally avoid stress (9%). Further it is clear, that people living with SCD are motivated to try a new therapy that could provide “significant relief” and “prevent symptoms from happening” due to their SCD.

Summary/Conclusions: The survey collected feedback about topics for which the patient is the best source of information. It is obvious that people with SCD are willing to self-medicate by subcutaneous injections and that there is a need for new tools and medications. With support from the answers from the survey, specific aspects will be considered while designing a first clinical study for subcutaneous sevuparin/DF02 administration to treat early symptoms of painful crisis in an at-home setting.

Table 1.

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 crisis 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 outcome will provide important information and, in combination with future feasibility studies, will guide the design of the first clinical study aimed at treating the early symptoms of pain crises in SCD patients.

Methods: A 29-question survey was created to gather input on a wide variety of topics related to the lives of people living with SCD. This questionnaire was developed by Modus Therapeutics AB, Sweden, in conjunction with Micromattie Consulting Inc., USA. Experts and leaders of community-based organizations participated in two focus group sessions to ensure that the text and structure were ethical and appropriate for the intended purpose. The survey was hosted at www.modustspatientsurvey.com. Patients answered the survey directly, or had their views entered in by a caregiver. The answers are anonymous. During the initial period, survey promotion occurred within the Sickle Cell Warriors online community and later, additional connections within the network of community-based organizations were leveraged. The survey was open for access during the period of January 10, 2017 through March 1, 2017.

Results: An interim analysis was conducted on January 31, 2017. Basic demographic data is presented in Table 1. Responders were located mainly in the US. Medical history related questions indicate that fatigue (40%), aches/pain (37%), irritability (27%) and appetite (20%) are early symptoms and increase just before the onset of a pain crises. However, 7% reported infrequent signs and 19% never experienced an indicator of pain crisis. Patients take initiative

Figure 1.

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 THALASSEMA

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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, 0-0.83, 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 treatment 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±2.2pg, 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 (9.566±3.674/μl vs 7.466±3.460/μl, p=0.009) and PLT count (333,778/μl±170,227 vs 272,111±160,304/μl, p=0.007) was noted. Concerning adverse events, one patient presented with mild transaminasemia, one with elevation of serum creatinine levels and one with pancytopenia. Due to persistent pancytopenia HU treatment was discontinued in the last mentioned patient, but was restarted a year later due to frequent vaso-occlusive events - despite the patient being put on transfusions after initial HU discontinuation. Besides the pancytopenia episode, the rest of the mentioned toxicities were self-limited and dose-dependent.

Summary/Conclusions: The study indicates that HU has an overall safe profile and results in a marked improvement of clinical course in pediatric S/b thal patients.
IN VITRO AND IN VIVO EVIDENCES OF SICKLING REVERSAL INDUCED BY REHYDRATION WITH HIGH K+-ISOTONIC SOLUTION

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Background: Erythrocyte sickling and adhesion are favoured by cellular dehydration, which increases the rate of hemoglobin polymerization and cell sickling. Potassium channels include co-transport and calcium-activated potassium channel (Gar-
dos channel) mediate erythrocyte dehydration in sickle cell disease and β-tha-
lassemia. We investigated the in-vitro and in-vivo effects of various concentra-
tion 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 iso-
tonicity.

Aims: This study was aimed at ascertain the efficacy of high potassium isotonic solutions in rehydrating sickle cell and possibly reversing the sickness phenomen-
on at in vivo and in vitro situations

Methods: 1. Erythrocytes from twenty sickle cell anaemia (SCA) as well as 46
healthy subjects were studied. One part was treated with sodium metabisulphite
(Na2S2O5) solution to induce maximum sickle cells as well as the other was sub-
tected to different high concentrations of K+ in PSS as well as Cocos
nucifera water (40mM, 80mM and CNw - 65mmol/L respectively). The proce-
dure 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
Na2S2O5 (45%) were observed which decreased significantly (P<0.05, respec-
tively) to about 2% with Cocos nucifera and 10% with 80mM K+ PSS. The count
in 40mM K+PSS was not statistically significant. In both HB AA and SS subjects,
MCHC remained relatively stable when compared to the pre-ingestion sam-
tle (P>0.05, respectively) while MCH increased significantly in both
groups as early as 1hr and sustained till the 24th hour. MCHC was equally
raised in the in-vitro samples (P<0.05, respectively). The morphology of red cells
also indicated a lesser count of sickled red cells after the oral ingestion

Summary/Conclusions: Cocos nucifera water and other high potassium ion
solutions can activate the rehydration of sickled erythrocytes by probably
de-activating the Gardos channel to increase the mean corpuscular haemo-
globin concentration(MCHC) and thereby restoring the normal red cell shape.

Results: After ingestion of the Cocos nucifera water for 24h, we observed
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.

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-Nor-
mandie. The purpose of this work is to assess the status of vitamin D in children
with SCD and thalassemia between 1998 and 2016 and we have reviewed their vitamin D levels. We have
reviewed their vitamin D levels. We have

Background: Sickle cell anemia (SCA) is a genetic disease causing a severe
disease manifesting by painful crisis but which can also be marked by organ
complications. Mortality is still happening at a young age. Many of these com-
placements may be better taken care of if treated early. The best way to manage
this disease is probably through Patient Education (PE). The recent evidence on Sickle cell disease 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.

Summary/Conclusions: The study found a high prevalence of vitamin D defici-
ency in children older than five years old (in the first determination) with SCD
da diagnosis of Major and significant decrease of levels in those not having
vitamin D therapy. It is not well known the physiopathology of this factor defi-
cency, 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 an adequate 25(OH)D levels. Although we have observed that 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
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not only the vitamin levels will increase but bone growth also.
Background: Patients with sickle cell disease (SCD) may require repeated red blood cells (RBCs) transfusion, putting them at risk from minor blood group alloim­munity and the development of delayed haemolytic transfusion reactions. Anec­dotally, we reported a prevalence of 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. All patients received RBC transfusions before developing clinical signs of haemolysis to assess the severity of DHTR. Mortality rate in our series was low (zero). Pain episodes and other complications associated with DHTR were treated as required and four cases were successfully monitored in the HDU. One patient required noninvasive ventilations and inotropic support. Two patients received RBC transfusions following a 24/7 STAT analysis (median 1.5 unit of packed RBCs). Possibly as their presence 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 aetiologies are useful. Whenever a DHTR is suspected, further RBC transfusion should be withheld unless absolutely necessary, as it may precipitate acceleration of the haemolytic 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 DHTRs. The 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). For Hbs monitoring of SCD patients we used 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.

Methods: Within- and between-run imprecision were assessed using a sickle cell trait and a sickle cell anaemia sample, aliquoted and stored at -80°C, twice daily (10 replicates for ten days), performed using duplicate measurements of a dilution set of 11 samples (Hbs S: 0% - 88%). Additionally, a comparison study was conducted between TOSOH G8 and Minicap Flex Piercing using 32 whole blood left-over Hbs samples (Hbs S: 9% - 93%). Data analysis was performed using Microsoft Excel Analyze-it version 4.6.2.

Results: Within- and between-run imprecision were <2% and an acceptable linearity was observed. Passing-Bablok regression analysis comparing TOSOH G8 and Minicap Flex Piercing showed an acceptable correlation coefficient of 0.95 (p<0.05) and a slope and intercept of 0.94 (95% CI: 0.92-0.96). The bias was 0.057 (95% CI: -2.5 to 1.3), respectively. Differences in Hbs results between TOSOH G8 and Minicap Flex Piercing ranged from -8.76% to +0.36% (mean difference: -3.54%). 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 ITM.

PB2153
GENDER DIFFERENCES IN THE DEVELOPMENT OF CMR ABNORMALITIES AND CARDIAC COMPLICATIONS: A MULTICENTRIC PROSPECTIVE STUDY IN A COHORT OF SICKLE CELL DISEASE PATIENTS
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1Fondazione G. Monasterio CNR-Regione Toscana, Pisa, 2Presidio Ospedaliero “Giovanni Paolo II” - Distretto AG2 di Sciacca, Sciacca, 3Azienda Ospedaliero-Universitaria di Bologna - Policlinico “S. Orsola-Malpighi”, Bologna, 4Presidio Ospedaliero ASL 5, Crotone, 5Clinico Umberto I, Roma, 6Presidio Ospedaliero “Umberto I”, Siracusa, 7Azienda Ospedaliera “Garibaldi” Presidio Ospedaliero Nesima, Catania, 8Fondazione di Ricerca e Cura “Giovanni Paolo II”, Campobasso, Italy

Background: No data are available in literature about the relationship between gender and the development of CMR abnormalities and/or cardiac complications in sickle cell disease (SCD).

Aims: This prospective and multicentre study aimed to assess if there was an association between gender and risk of cardiac iron overload, heart dysfunction and dilatation, left ventricular (LV) hypertrophy, and myocardial fibrosis, assessed by Cardiovascular Magnetic Resonance (CMR), and of cardio-vascular complications in sickle cell disease (SCD) patients.

Methods: We considered 115 SCD patients (58 females, 34.79±13.26 years), consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Myocardial iron overload was assessed by the multivoxel multiecho T2* technique. Biventricular function parameters and atrial areas were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: Table 1 shows the comparison between sexes in the development of cardiac outcomes. Males and females showed a similar risk of accumulating cardiac iron, but both patients with cardiac iron were females. Compared to females, males showed a significant lower risk of developing LV hypertrophy, although having a similar risk for biventricular dilation and dysfunction and for myocardial fibrosis. No patients with less than 31 years developed LV hypertrophy and age at the CMR was significantly higher in patients with LV hypertrophy versus patients without it (41.24±75.98 years versus 34.47±13.37 years; P=0.003). We recorded 12 (10.4%) cardiac events: 4 ischemic strokes, 5 arrhythmias (4 supraventricular and 1 ventricular), two pulmonary hypertensions and one pulmonary embolism. No prospective association was detected between gender and cardiac complications. Table 1.
Background: Acute pain is a hallmark presentation in sickle cell disease (SCD) and frequently requires attendance to the emergency department (ED).

Methods: A retrospective analysis was performed with 1202 hemoglobin (Hb) studies at Hospital Universitario Central de Asturias between January 2006 and March 2016. The studies came from medical applications, the finding of anaemia 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.

Results: In 2014 the ED met CEM criteria for the timeliness of anaesthesia 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.

PB2154

SICKLE CELL PAIN IN CHILDREN: TARGETS FOR ADMINISTRATION OF ADEQUATE INITIAL ANALGESIA

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Results: 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.

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PB2155

DIAGNOSTIC CHALLENGES IN A POPULATION WITH INCREASED IMMIGRATION: HEMOGLOBINOPATHIES IN THE NEW CENTURY

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Results: We analyzed 1202 patients, 49% were males and the median age was 33 years (range 0-85). We found 562 patologic studies. - Thalassemia were detected in 393; 337 were β or δ (86.4%); 54% came from Spain. The cases of β-thalassemia were: 5 intermedia, 3 major, 1 δ-hemoglobin and the remainder were minor (97%). All thalassemia major, 3 intermediate and 74% of minor were Caucasian. Anemia was found in all of major thalassemia (median Hb 6.2 g / dl, range 6-6.5), in 3 of intermedia (11.4 g / dl, range 6-10), and in 197 of minor (10.87 g / dl, range 6.9-11.9). - Structural Hbts were found in 170, the predominant was Hb S (n=125). Only 17 sickle cells (HB SS and HB SC). Most of them black (n=14) coming from Africa (n=10) and South America (n=4). Eighty six percent (n=108) were heterozygous (HB SA), mainly from Africa (n=56) and South America (n=23). Anemia were seen: 4 Hb SC (median Hb 10.5g / dl, range: 9.4-11.2), 9 Hb SS (9.74g / dl, range 5.2-9.7) and 37 heterozygotes (10.15g / dl, range 6.7-11.9). There are two peaks of higher incidence of structural Hbts, in 2008 coinciding with the creation of the Tropical Diseases Unit and since 2013 when detection increases with the introduction of HbA1c test. The increase in thalassemias was due to the decision to extend studies due to pathological findings in hematimetry results (Figure 1).

Figure 1.

Summary/Conclusions: In our area there is a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts. In our area the diagnosis of Hbpts is a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts.

PB2156

EFFECT OF SUSTAINED-RELEASE SUPPLEMENTATION OF L-ARGININE AMONG CHILDREN WITH SICKLE CELL DISEASE IN FEDERAL TEACHING HOSPITAL GOMBE, NORTH EASTERN NIGERIA

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Methods: This study included children with sickle cell disease (HbSS) aged 1-14 years with mean age 7.4500±0.50613 years presenting to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. Subjects received sustained release oral L-arginine supplementation of 350mg twice daily for 8 weeks.

Results: L-arginine and nitric oxide levels were significantly higher among sickle cell disease children. There were no statistically significant differences between the baseline and post L-arginine supplementation in the PCV, WBC, RBC and LYM levels of subjects (p>0.05). There was a statistically significant difference between the baseline and post L-arginine supplementation in the MCV, MCH, MCHC, PLT, NEU, EOS, MON and RDW-SD levels of subjects (p<0.05). The L-arginine and nitric oxide levels were significantly higher post supplementation compared to baseline levels (p=0.002 and 0.000 respectively). The Total Antioxidant Capacity was significantly higher post supplementation compared to baseline levels among subjects with vaso-occlusive crisis (p=0.001, 0.01 and 0.05 respectively). The panthothenic acid and malondialdehyde levels at baseline were significantly higher than the post supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively). The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among subjects with vaso-occlusive crisis (p<0.05 and 0.000 respectively). The baseline plasma malondialdehyde level was significant higher that the post supplementation levels among the sickle cell disease subjects. There is need for more effort and resources to be dedicated to research especially in supplementation studies involving a larger population aimed at establishing specific treatment for sickle cell disease. It is recommended that L-arginine supplementation be included in the management of patients with sickle cell disease particularly those with vaso-occlusive crisis. We observed a statistically significant negative correlation between the L-arginine levels and the red cell count among sickle cell disease subjects (r=-0.350, p=0.043).
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, malondialdehyde 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 disease-free, 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% (30.1-38.9%) 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% (33.3-55.8%) 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% 1.19-2.78, p<0.001), underweight (<5th percentile, HR=1.6, CI 95% 1.17-2.16, p=0.001 and HR=1.34, CI 95% 1.04-1.72, p=0.02, respectively). Positive CMV serology (HR=1.5, CI 95% 1.01-2.33, p=0.03) was associated with higher NRM. ATG use (HR=1.6, CI 95% 1.05-2.31, p=0.03) was associated with higher relapse incidence. Moreover, ATG use and a positive CMV serology were associated with worse OS (HR=1.6, CI 95% 1.5-2.17, p=0.04 and HR=1.3, CI 95% 1.01-1.69, p=0.001, respectively) and LFS (HR=1.6 CI 95% 1.17-2.16, p=0.001 and HR=1.34, CI 95% 1.04-1.72, p=0.02, respectively). Infused TNC >4.2x10^7/Kg (HR=1.5, CI 95% 1.07-2.14, p=0.02), lack of ATG in the conditioning (HR=2.72, CI 95% 1.6-3.1, p<0.001) and BMI <5th percentile (HR=1.8, CI 95% 1.19-2.78, p=0.001) were associated with higher incidence of acute grade II-IV GVHD.

Summary/Conclusions: In conclusion, we did not find association of obesity with transplant outcomes in this study population. However a BMI <5th percentile at UCBT was found to be associated with higher risk of acute GVHD, highlighting the importance of nutritional status before UCBT.
PB2158

PROSPECTIVE PHASE STUDY OF REDUCED TOXICITY CONDITIONING CONSISTED OF HIGH DOSE CYTARABINE, FLUDARABINE, CYCLOPHOSPHAMIDE +/- TOTAL BODY IRRADIATION FOLLOWED BY 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, to 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. Preliminary treatment 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 days of TBI was used for all CB transplant recipients, whereas 2 gray of TBI was used in other stem cell sources exclusively according to each institutional policy. Calcineurin inhibitors (cyclosporine or tacrolimus) and short term methotrexate were used as GVHD prophylaxis. Donor cell engraftment and 60 day-survival were assessed as a primary end point to evaluate feasibility of this protocol.

Results: Thirty nine patients including 7 recipients with a history of SCT were enrolled. Median age was 61 (28-69), 21 were male, and 18 were female. Nineteen were acute myeloid leukemia, 11 myelodysplastic syndrome, 6 malignant lymphoma and 3 acute lymphoblastic leukemia. Donors were 4 matched related, 8 unrelated, 2 mismatched related, 1 second degree, and 22 s2-Ag-mismatched CB. Thirty seven (94.9%) patients have passed 60-day-point 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 observed up to 1 year. Overall survival and disease-free survival were estimated to be 82.1% and 73.8% at 1 year post-transplant, respectively. Median neutrophil recovery to over 500/μl was observed up to 1 year. Overall survival and disease-free survival were estimated to be 82.1% and 73.8% 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 are required to evaluate the eventual survival benefit by reducing relapse.

PB2159

LATE COMPLICATIONS OF CONDITIONING REGIMENS (CYCLOPHOSPHAMIDE - TOTAL BODY IRRADIATION vs BEAM) FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN LYMPHOMA.

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Background: Autologous stem cell transplantation (ASCT) is a frequently used procedure for the treatment of patients with relapsed non-Hodgkin lymphoma (NHL). While chemotherapy-based regimens are now commonly administered, total body irradiation (TBI) was largely used in the past. The current conditioning regimen in our center is BEAM (a combination of carmustine (BCNU), etoposide, cytaraabine and melphalan) although we also have a large experience with cyclophosphamide (CFM)-total body irradiation (TBI) since this was the usual conditioning until year 2000.

Aims: To analyze the cumulative incidence of secondary neoplastic complications (grade 3-4 infections, cardiovascular and pulmonary toxicity) after the two conditioning regimens (CFM-TBI vs BEAM) for ASCT.

Methods: We performed a retrospective analysis of patients with NHL that 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% (95% CI 86-90% 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.

Table 1. Patient’s characteristics.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Frequency (%)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>51-60</td>
<td>51.1</td>
<td>61.3%</td>
</tr>
<tr>
<td>61-70</td>
<td>48.9</td>
<td>38.7%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
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</tbody>
</table>

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: DONOR LYMPHOID INFUSION AND BREN’TUXIMAB.

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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 transplantation (alloSCT) can be a second line treatment. 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 standard-ized treatment for this situation.

Aims: To analyze the outcome of post-transplant relapse treatment of haploidon donor haemato poetic progenitors (haploTPh).

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.
Methods: We studied 127 adult patients who underwent ASCT following LEED or MEC as the conditioning regimen against chemo-sensitive ML at four institutions in Japan between 1997 and 2015. Any type of pathologic diagnosis was considered. The LEED regimen consisted of 140 mg/m² L-PAM (day −1), 500 mg/m² etoposide (days −4 to −2), 60 mg/kg cyclophosphamide (days −4 to −3), and 40 mg/body dexamethasone (days −4 to −1). The MEC regimen consisted of 200 mg/m² MEL (days −3 and −2), 300 mg/m² carboplatin (days −7 to −4), 500 mg/m² etoposide (days −6 to −4), and 50 mg/kg cyclophosphamide (days −3 to −2). Fisher’s exact test was used to compare binary variables. OS rates were estimated by the Kaplan-Meier method. TRM was compared using the stratified Gray test. Cox proportional hazards regression model was used for multivariate analysis of OS. Values of p < 0.05 were considered significant.

Results: Of the 127 patients, 76 were male and 51 were female, and the median age at diagnosis was 60 years (range: 18 to 86 years). 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% of the patients received the LEED regimen and 19% the MEC 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 MEC 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 MEC groups (77% vs 88%; p = 0.35). Likewise, both the 5-year CI of relapse and NRM were similar in the two groups (17% vs 16% and 5% vs 5%, respectively). The analysis 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 observed in 3% of the patients. In grade 3 or 4 limb incidents, 36% (n = 51) and 4% (n = 7), respectively. In grade 3 or 4 GI incidents, 28% (n = 40) and 5% (n = 7), respectively. The incidences of grade 3 or 4 neutropenic infections were 4% (n = 6) and 1% (n = 2), respectively. The patients who relapsed and in 70% (7) in the non-relapsed group. 38% of the whole group of patients, had a donor/recipient KIR allorreactivity without differences between the two groups of the study. 88% (7) of the relapses occurred before 6 months of the SCT. The mean time to relapse was 316 days (range 181-446). Between the 8 relapsed patients and 9 patients who received another center with Vindablastine / Dexamethasone and by infection, another patient died by abdominal sepsis before starting any treatment. Brentuximab was administered in 63% (5) of the patients. One of them received a single Brentuximab cycle with no tolerance, and changed to RT, GDP+Donor lymphocyte infusions (DLI) and had reached complete remission after 5 DLI. The rest (4) received between 3 and 7 doses with adequate tolerance. According to the re-evaluation (PET-TC) after 3rd Brentuximab, 4 were in partial remission and one reached complete response. We associated Donor lymphocyte infusion in 6 patients. The mean of DLI received was 10; the median was 8, with a range between 22-3. All patients reached complete remission, two of them maintain a partial response. All of them presented good tolerance to DLI. We observed Graft versus host disease in four patients, three of them presented moderate cutaneous affection, and one of them suffered hepatic graft versus host disease stage III, with adequate evolution after treatment. Survival Conclusions: Is possible to treat patients who relapsed after haploidentical stem cell transplantation with Brentuximab+DLI, with a very good tolerance. We observed cutaneous graft versus host disease in most of the patients who reached complete remission after DLI. Despite this findings, we need multicentric studies to perform standardized treatments and protocols.

PB2162
ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR SECONDARY HAEMATOLOGICAL NEOPLASIA: A SINGLE CENTER EXPERIENCE
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Background: Therapy related haematological neoplasms (t-HN) occur due to direct mutational events of chemotherapeutic agents and radiotherapy. Disease latency, mutational events and prognosis vary with drugs categories.

Aims: The aim of this retrospective study was to assess the outcome of t-HN and hematopoietic stem cell transplantation (HCT) in these patients.

Methods: We describe a cohort of 31 patients, 19 females (61.3%) and 12 males (38.7%), with median age of 53 years (range, 20 to 64), who received an allogeneic HSCT in our Unit, between September 1999 and July 2016. Patients had a history of solid tumor in 15 cases (48.4%), haematological malignancies in 15 cases (48.4%) and both of them in one case (3.2%). All but one received a median of 2 (range, 1 to 6) lines of therapy. After a median of 36 months (range 12-190) from the first neoplasia, patients developed t-AML (n=19) (61.3%), t-Ph+ ALL (n=1) (3.2%), t-MDS (n=11) (35.5%). Molecular abnormalities were detected in 7 (46.7%) out of 15 evaluable cases: 12q- (3), t-Ph+ (3), t-MDS (1). The conditioning regimens were reduced intensity conditioning (RIC) in 14 cases (45.2%), high intensity conditioning (RIC) in 11 cases (33.3%), reduced intensity regimens based on conditioning regimens from ASCT and MEC in 4 cases (12.5%). A total of 21 patients (67.7%) had at least one prior transplant. A karyotypic aberration was found in 18 (64.3%) of the 28 evaluable patients: 16.7% was favourable risk (n=3), 27.8% was intermediate risk (n=5) and 55.5% was adverse risk (n=10). The disease status at transplant was as follows: complete remission (n=13) (42%), refractory disease (N=10) (32%), relapse (4%) (n=3) (10%). Patients received conventional chemotherapy in 14 cases (45.2%), azacitidine in 11 cases (33.3%), both of them in one case (3.2%), whereas 5 patients (16.1%) were untreated. The conditioning was myeloablative (MAC) in 20 patients (64.5%) or reduced intensity (RIC) in 11 patients (35.5%); the donor was a family member (REL) in 17 patients (54.8%) or unrelated (MUD) in 14 patients (45.2%). The hematopoietic cell transplantation comorbidity index (HCT-CI) was as follows: 14 patients (45.2%) had a score of 3 and 17 patients (54.8%) had a score of 4 or more. Overall survival was calculated with Kaplan-Meier method. Transplant-related mortality (TRM) and relapse-related mortality (RRD) rates were estimated by competing risk models considering the opposite event as competing. Fine and Gray’s method for CI of TRM and RRD was used to evaluate the risk factors on univariate analysis.

Results: Twenty-three patients were in remission on day +30, by bone marrow cytology, 3 patients were classified as resistant disease and five patients were not evaluable. TRM was not observed because of early death. Five patients (21.7%) relapsed after a median of 6 months (range, 3 to 15). At the time of this analysis (December 2016) 14 patients were alive with a median OS of 53 months (range 8-190), while 17 patients died after a median of 4 months (range 1-27): RRD was 16% (n=5) and TRM was 39% (n=12). Non relapse causes of death were as follows: GVHD (n=3), infections complications (n=8) and EBV-related PTLD (n=1). One patient experienced a third tumor (breast cancer) thirteen years from HSCT. TRM was higher for patients transplanted from MUD (66%) as compared to REL donor (16%) (p=0.01). The overall survival was 45.2% (Figure 1) and 58% maintained a complete remission.

Summary Conclusions: This report confirms that allogeneic HSCT is a curative approach in approximately 50% of patients with therapy related haematological neoplasms, especially for those patients who benefit from a familial donor.
PB2163
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 myeloma ablative 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 EchoclinigntCH. Chart review was conducted to determine association of significant renal dysfunction, 2D-echo and strain imaging offer non-invasive modalities for identifying early cardiac changes independent of renal function.

Results: Of the 7 patients studied, 3 were Mayo stage I, 2 stage II, 1 stage III, and 1 stage IV. The median follow-up from transplant was 47.4 months. There was one death at 20.4 months. The mean NYHA classification at baseline was 2.3 and after transplant was 1.9. Longitudinal, radial and circumferential left ventricular strain (LV) were evaluated, but only the global longitudinal strain (GLS) showed an improvement (baseline -14.69%; follow-up -16.84%; mean absolute improvement 2.15%; p <0.05) across all four Mayo Stages. There was no difference in GLS within individual stages. In patients with stable NYHA classification after treatment 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 was 1.9. Longitudinal, radial and circumferential left ventricular 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.

Summary/Conclusions: We demonstrate that there is a clinically meaningful improvement in cardiac mechanics one year after Auto-HCT, despite no alteration in LVEF. This metric may prove useful in assessing organ response, especially when serum biomarkers are less reliable. Changes in left ventricular GLS occur independent of pre-transplant Mayo stage, although prospective studies are needed for confirmation. We further believe that improvements in RVFWS may predict clinical improvement.

PB2164
AN ABSOLUTE NUMBER OF CD34+ CELLS IN BLOOD AS A PREDICTOR OF A SUCCESSFUL HARVEST OF HEMATOPOIETIC STEM CELLS IN DIFFERENT MOBILIZATION REGIMENS
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Background: Autologous stem cells transplantation (ASCT) has become necessary part in therapy of hematological diseases. Transfusion of at least 2×10^6 CD34+ HSCs per kg of patient’s weight allows achieving an adequate hematopoiesis after high-dose chemotherapy. The most optimal is to collect ≥2×10^6 CD34+ cells/kg with single harvest apheresis. Different mobilization regimens lead to variations in white blood cell count (WBC) and the number of circulating HSCs. Deciding on the time of the first leukapheresis is very important.

Aims: The aim was to identify significant parameter predicting success of CD34+ cells collection.

Methods: The study included 142 patients (pts) who undergoing ASCT (80 m, 62 f, median age 53 y.o.; 81 were diagnosed with multiple myeloma, 10 - Hodgkin’s lymphoma, 51 – non-Hodgkin’s lymphomas). WBC and absolute CD34+ number in the blood before the first apheresis and the number of CD34+/kg in the apheresis product were determined for each patient. There were three different mobilization regimens: 1) 28 pts received 10 μg/kg/day G-CSF for 5-6 days; 58 pts - 4 g/m² cyclophosphamide and 5-10 μg/kg/day G-CSF (Cph+G-CSF); 44 pts - DHAP: 40 mg dexamethasone, 100 mg/m² cisplatin, 2g/m² cytarabine and 10 μg/kg/day G-CSF (DHAP+G-CSF). CD34+ HSCs were evaluated with ISHAGE-protocol by BD FACSCanto II flow cytometer. Results are presented as meanSEM. ROC-curve analysis was performed for WBC and the absolute number of CD34+ HSCs in the blood as the predictor markers for HSCs successful harvesting (≥2×10^6 CD34+/kg for fist apheresis).

Results: WBC was higher in pts with G-CSF mobilization scheme compared to Cph+G-CSF and DHAP+G-CSF (28.5±3.5 vs 10.4±0.9 and 9.0±1.8×10⁹/l, respectively, p<0.0001), but the absolute number of CD34+ HSCs in the blood was 29 CD34+cells/μl in G-CSF mobilization, 24 CD34+cells/μl – in Cph+G-CSF and 27 CD34+cells/μl – in DHAP+G-CSF. The ROC-curve analysis showed the optimal CD34+ number in blood in the most likely to collect ≥2×10⁶ CD34+/kg for first leukapheresis. It was 29 CD34+cells/μl in G-CSF mobilization, 24 CD34+cells/μl – in Cph+G-CSF and 27 CD34+cells/μl – in DHAP+G-CSF. To calculate universal level of absolute CD34+ number all data from 142 pts was used. In this case AUC was 0.952 and a threshold of successful harvesting was 20 CD34+ cells/μl in blood before apheresis with sensitivity of 96% and specificity of 81%.

Summary/Conclusions: Various mobilization regimens differ in count of leukocytes and CD34+ HSCs in peripheral blood: WBC was significant higher in G-CSF than in Cph+G-CSF and DHAP+G-CSF, but the absolute number of CD34+ cells was higher in chemotherapy-based mobilization and G-CSF than in Cph+G-CSF alone. The absolute number of leukocytes in blood before apheresis was not a predictor factor of harvest success in all variants of mobilization regimens. If there is at least 20 CD34+cells/μl in blood before apheresis it is possible to collect ≥2×10⁶ CD34+/kg for single leukapheresis with high sensitivity and specificity independent of mobilization regimen.

PB2165
QUANTIFICATION OF CD34+ CELL AND ITS VIABILITY OF FRESH OR CRYOPRESERVED NUCLEATED CELLS BY IMAGE-BASED CELL COUNTER IS COMPARABLE TO STANDARD FLOW CYTOMETER
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Background: As a standard method for quantification of CD34+ stem cells, flow cytometry has been widely used. However, it has some limitations such as...
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 used samples of fresh and cryopreserved nucleated cells from G-CSF-mobilized peripheral blood stem cells (PBSCs) as well as cord blood (CB). We assessed the reproducibility and linearity of the new device and compared numbers and viabilities of CD45+ cells and CD34+ cells determined with the ADAM II™ and flow cytometer.

Results: Each analysis used 10 aliquots from one sample to assess the reproducibility. Each analysis was repeated 14, 177-172.06 CD34+ cells (0.08-0.56 CD34%/CD45). The number of CD34+ cells determined by ADAM II™ was sufficiently accurate over the expected range, and the intra-assay coefficient of variation (CV) was ≤10.8%. The linearity of CD34+ cell count was confirmed over a range of dilutions (0.59-280 cells/ml) of sample. Linearity was ≥99.5%. The numbers and viabilities of CD45+ cell and CD34+ cell obtained with the ADAM II™ were highly correlated with those obtained with the flow cytometer (R²=0.9841, P<0.0001). In all samples from fresh/cryopreserved PBSC and fresh/cryopreserved CB, there were no significant differences in total numbers and viabilities of CD45+ cell and CD34+ cell counts determined by flow cytometer.

Summary/Conclusions: The newly developed image-based microscopic cell counter (ADAM II™) appears to be suitable for quantification of CD34+ cell and its viability of fresh or cryopreserved PBSCs or CBs.

PB2166

EXTRACORPOREAL PHOTOHERESIS IN STERIOD-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 (allo-HCT) in many centres. The introduction of ECP as an early second-line treatment in steroid-dependent or refractory patients with acute GVHD (aGVHD) remains under study. The rationale of its early use is based on the low incidence of complete responses to corticosteroids and the profound immunosuppression caused by traditional secondary treatments.

Aims: Based on our long-lasting experience in chronic GVHD, we aimed to prospectively assess the role of ECP in this high-risk population.

Methods: We enrolled consecutive patients with steroid-dependent or refractory grade (gr) II-IV aGVHD post allo-HCT from January 2013 to August 2016. All patients with unrelated or haploidentical donors received thymoglobulin (ATG) 5mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included cyclosporine – metotrexate in myeloablative and cyclosporine – mycophenolate mofetil in reduced toxicity or intensity regimens. ECP was commenced after assessment of response to 5 days of steroid treatment according to our protocol: 2 sessions/week for 1 month, 1 session/2 weeks for 3 months, evaluation of response and 1 session/month for 6 months.

Results: We studied 20 patients, aged 35 (18-65), post allo-HCT with myeloablative (14), reduced toxicity (4) and intensity (4) conditioning, from sibling (8), matched (8) or one locus mismatched (8) volunteer unrelated and haploidentical (1). 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 refractory patients that further developed EBV reactivation (p=0.032) treated in 6 refractory patients that further developed EBV reactivation (p=0.032). Nine patients (2 with GrIV) steroid-refractory. ATG was administered simultaneously with ECP initiation (ATG) 5mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included steroids and the profound immunosuppression caused by traditional secondary treatments.

Summary/Conclusions: These results indicate that rapid reconstitution of NK cells; especially NK1 cells would be help to prevent the development of graft-versus-host disease as well as CMV reactivation after allogeneic transplantation.

PB2167

RAPID RECONSTITUTION OF NK1 CELLS IS ASSOCIATED WITH THE LOWER INCIDENCE OF GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC TRANSPLANTATION

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Background: The balance between immunostimulation and immunoregulation in T cell immunity is achieved by a Th1/Th2/Th3/T1r and CD4+CD25+ regulatory T (Treg) cell paradigm.

Aims: We investigated the production of type1 (IFN-gamma, NK1), type2 (IL-13, NK2), type3 (TGFBeta, 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 reconstructed 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 0.015, 0.005-0.348, P=0.003).

Summary/Conclusions: Our study supports that ECP should be considered early in the treatment of patients with severe steroid-refractory aGVHD, because rapid NK1 cell reconstitution is associated with lower incidence of GVHD.

PB2168

BORTEZOMIB FOR STEROID-REFRACTORY RITUXIMAB AUTOIMMUNITY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Therapy of post-transplant autoimmunity manifestations remains a challenge. Many cases are steroid and rituximab refractory and continuing intensified immunosuppression increase the risk of infection in the post-HSCT patient. In our institution, we have used bortezomib as our third agent after failure of steroids or rituximab, or in cases of steroid-dependence since Bortezomib appears to be effective in cases with refractory autoimmunity.

Aims: In our series, we assessed the therapeutic response to proteasome inhibitor in 4 cases of post-transplant refractory autoimmunity.

Methods: Three of the 4 patients received Bortezomib for autoimmune cytopenia (autoimmune haemolytic anaemia AIHA (n=2), AIHA with acquired red Cell Aplasia (n=1)). At least 2 therapy lines had failed to resolve the cytopenia. One to two courses of Bortezomib were administered at a dose of 1.3 mg/m2 at day 1, 4, 8, 11 each course. In two cases this treatment was combined with immunosuppressive agents: Mycophenolate mofetil (MMF) alone in one case and associated with sirolimus in the other case.

Results: Resolution of autoimmune cytopenia was observed in the three cases after a median of 33 days from the first day of administration. The fourth case received 1 course of Bortezomib for persistent anti-enzyme antibodies after allogeneic transplant for Wolman disease. Therapeutic response was obtained after 25 days reflected by a complete regression of circulating anti-enzyme antibodies. In all cases, no Bortezomib related toxicity was noticed. The response was maintained in all cases.

Table 1 summarizes the clinical data and the results of the four cases.

Table 1.
POST-THAW CELL COUNT PREDICTS ENGRAFTMENT RATE IN CORD BLOOD TRANSPLANTATION

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Background: The infused cell count in cord blood transplantation (CBT) is an important element for engraftment; however, this number in the prior reports has been based on the pre-thaw cell count. Therefore, the association between post-thaw cell count and engraftment rate, especially in pediatric patients, is unclear.

Aims: The aim of this study is to reveal the association between post-thaw cell count and engraftment rate in pediatric patients in the setting of CBT at our institution.

Methods: We retrospectively reviewed the medical records of 78 patients who underwent CBT between June 1998 and April 2016. We excluded the cases of CBT that required rescuing after engraftment failure.

Results: Underlying disease was acute leukemia (AL) in 63 (ALL, 38; AML, 25) patients, chronic myeloid leukemia in one, malignant lymphoma (ML) in two, myelodysplastic syndrome (MDS) in three, aplastic anemia in one, and others (such as primary immunodeficiency syndrome) in eight. In terms of conditioning regimens, myeloablative conditioning was administered to 62 patients and reduced intensity conditioning was administered to 16 patients. The median age at CBT was 3 (range, 0–19) years, and the median follow-up period was 898 (range, 47–6236) days. The engraftment rate was 84.6%, primary engraftment failure was observed in 11 patients (AL seven; ML, one; MDS, one; neutroblastoma, one; and others, one) and secondary graft failure was observed in one patient (severe congenital neutropenia). 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 was 1.60 × 10^5/kg in the patients who achieved engraftment and 1.01 × 10^5/kg in the patients who did not achieve engraftment. No statistically significant difference was observed between these two groups (p=0.30). When we defined the cut-off value of the pre-thaw CD34+ cell count as 1.2 × 10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 79.3% and 60%, respectively. When we defined the cut-off value of the post-thaw CD34+ cell count as 0.7 × 10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 96.6% and 40%, respectively.

Summary/Conclusions: We concluded that the risk of graft failure is more precisely predicted by the post-thaw than the pre-thaw CD34+ cell count and that the cut-off value of CD34+ cell count as 0.7 × 10^5/kg, the risk of graft failure is very low.

URIC ACID LEVEL MIGHT BE A PROGNOSTIC INDICATOR FOR SURVIVAL IN PATIENTS WHO UNDERWENT ALLOGENIC 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 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, creatine, total protein and albumin were analyzed. Univariate, multivariate Cox regression models and Kaplan-Meier survival analysis were performed. To uric acid, creatine, total protein and albumin associated with disease-free survival (DFS) were all survival (OS), early non relaps mortality (+30 day) and late non relaps mortality (+100 day).

Results: Pretransplantation low uric acid levels were detected in 57 (32.08) percent patients. Low UA levels were significantly associated with DFS (HR=0.52; p=0.027). None of the creatine, total protein and albumin were significantly associated with DFS (HR=0.98; p=0.98, HR=0.87; p=0.60, HR=1.15; p=0.66). There was no significant association between UA, creatine, total protein and albumin and survival. UR levels in OS survival analysis were not significant. Survival analysis were not significant and late non relapse mortality (HR=0.57; p=0.35, HR=0.21; p=0.29, HR=0.94, HR=0.94, HR=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 our inability to catabolize UA due to our inability 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.

Background: Herpes simplex virus type-1/2 (HSV-1/2) can reactivate after allo- genic hematopoietic stem cell transplantation (allo-HSCT) even when the prophylactic acyclovir is used. However, the risk factors for HSV-1/2 viremia and the clinical outcomes following unmanipulated haploidentical HSCT remain unknown.

Aims: The aim of this study was to explore the risk factors for HSV-1/2 viremia and to evaluate clinical outcomes following haplo-HSCT.

Methods: Nineteen patients with HSV-1/2 viremia and fifty-seven patients without HSV-1/2 viremia were selected using the case-pair method after haplo-HSCT were enrolled. We analysed the risk factors for HSV-1/2 viremia and compared clinical outcomes between the two patient groups.

Results: The risk factors for HSV-1/2 viremia included HLA disparity ≥2 loci (p=0.049) and cytomegalovirus (CMV) reactivation (p=0.028). The incidences of platelet engraftment, oral mucositis and severe haemorrhagic cystitis (HC) in patients with and without HSV-1/2 viremia were 77% and 94% (p=0.003), respectively.
78% and 13% (p=0.000), and 25% and 6% (p=0.04), respectively. Moreover, the median time to platelet engraftment in patients with and without HSV-1/2 viremia was 25 d (range, 11–80 d) and 17 d (range, 8–67 d) (p=0.004). In a multivariate analysis, HSV-1/2 viremia was associated with delayed platelet engraftment (p=0.038), a higher incidence of oral mucositis (p=0.000) and severe HC (p=0.038). However, HSV-1/2 viremia was not associated with non-relapse mortality (31.5% 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).

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 strongly associated with high NRM even with RIST. This indicates that we should pay more attention to NRM in MDS.

INCIDENCE AND RISK FACTORS FOR THE DEVELOPMENT OF HEMORRHAGIC Cystitis on HAPLOIDENTICAL TRANSPLANTATION

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Background: Hemorrhagic cystitis (HC) is a serious complication occurring after alloegenic hematopoietic stem cell transplantation (HSCT) more frequent on haploidentical (haplo) HSCT, with an incidence of 10% to 70% (Silva et al Haematologica 2010;95(7):1183–1190) associated mainly with the effect of cytotoxic agents such as Cyclophosphamide (Cy). The conditioning regimen, BKPyV infection and graft versus host disease have an implication in the incidence. Other authors related the reactivation of CMV and a previous transplantation as risk factors to HC development (Ruggen et al Transplant Infectious Disease 2015;17:822–830).

Aims: With this study we aim to describe the HC incidence and risk factors in all haplo-HSCT performed in the Canary Islands.

Methods: We analyzed all consecutive haplo-HSCT from family donors performed at our Hospital between 2013 and 2016. The conditioning 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 last dose at 6, 8 and 24 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 immunosuppression state of the patient, we also observed all these pts had positive serum viral load for CMV.

Summary/Conclusions: The incidence of HC associated to post-HSCT high Cy dose in our series is 15% lower than other ones. Most of them on grade 1 or 2 and without mortality associated. The risk of HC is high, particularly in the setting of highly pre-treated patients (especially those undergoing a 2nd transplant). The development of HC after day +30 is evidently associated to BKPyV as a contributing factor for continuous inflammation and CMV reactivation (as an immunosuppression marker). In our study, HC did not have an impact on the development of the disease in high-risk patients after haplo-HSCT. The HC remains frequent with a high morbidity in particular when it is severe, often causing prolonged hospitalization and resource use. We need further studies to recognize the at-risk population early.
Background: Although the Bruton tyrosine kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of CLL patients (pts), allogeneic hematopoietic stem cell transplantation (HCT) remains the only curative option for the underlying disease. Data on pre-transplant treatment of CLL with ibrutinib are very limited. Disease status at HCT was Binet B (n=3) or Binet C (n=8). Two pts had Richter's transformation (RT) diagnosed before nma-HCT. Ten pts were in partial remission (PR) at nma-HCT (PR1 n=4; PR2 n=3; PR3 n=2, PR4 n=1) while one was in first relapse. Donors were human leukocyte antigen (HLA) matched related (n=3, MRD) or HLA-matched unrelated (n=8, MUD). Pts received median 3 lines of therapy (range 1-6) including ibrutinib before transplantation. Classical cytogenetic analysis and fluorescence in situ hybridization (FISH) was carried out for every pt. Five pts had a deletion (del)(17p13) and one a del(11q22.3).

Results: The average overall survival (OS) for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 465 days (range 36-812) for those without del(17p13), p=0.98. OS was not significantly influenced by the stem cell source (MUD vs MRD, p=0.63) or remission status PR1 vs >PR1 (353 vs 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn't reach significance (p=0.39). EFS was median 125 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p=0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our group (30% at 4y). The acute GVHD Grade 3-4 was present in 3 pts (27.2%).

Summary/Conclusions: Impact of chimerism in different T-helper subsets still need further investigation. We will continue our research and further results will be reported later.
differences of 3.9±4.9 cm and 2.8±4.4 cm in males and females, respectively (p<0.01). VAT/TAT witnessed a slight increase in males and reduction in females (p<0.05). In multivariate analysis, no significant associations were shown with mortality and progression rates (Figure 1).

Summary/Conclusions: This study provides data on the evolution of adipose tissues parameters in the peri-transplantation phase. VAT, VAT and WC decrease 3 months post transplantation. Future studies should evaluate the associations of these parameters with major outcomes on larger sample sizes.

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 NRM and, in the past decade, has been accepted worldwide as an alternative treatment for patients with hematological malignancies who do not have an HLA-identical sibling donor or who require urgent transplantation.

Aims: The purpose of this study was to investigate the incidence, causes and factors influencing overall and transplant-related mortality after Haplo-HSCT. 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 and, in the past decade, has been accepted worldwide as an alternative treatment for patients with hematological malignancies who do not have an HLA-identical sibling donor or who require urgent transplantation.

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 and, in the past decade, has been accepted worldwide as an alternative treatment for patients with hematological malignancies who do not have an HLA-identical sibling donor or who require urgent transplantation.

Figure 1.

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 80%. 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-
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 hematopoiesis, and the parameters of the infused product (CD34+ cells and 

Results: A total of 65 PBSC samples were collected. We compared three groups based on the cryopreservation period: (1) less than 6 months, (2) 7–24 months, and (3) 25–36 months. The median (range) viability of CD34+ cells after thawing was 81.8% (59.2–94.4), 80.5% (56.6–92.8), and 76.1% (54.5–89.6) in the three groups, respectively. No significant difference in the viability of the cells in either frozen period was observed (p=0.14, respectively). We compared the effect of granulocyte concentration (over 10% concentration against less than 10% concentration) on CD34+ cells viability. The median (range) viability of CD34+ cells containing >10% granulocytes was 76.6% (54.5–93.0%), and that for cells containing <10% granulocyte was 82.1% (59.1–94.4%), respectively. There was significant difference in the viability of CD34+ cells between the two groups (p=0.02, respectively). Second, we analyzed 81 autologous PBSC transplants after stored at -80°C by using simplified method. We compared the two groups based on the granulocyte concentration (10% concentration against <10% concentration). No significant difference in the days to leukocyte >1x10^9/L and to platelet >20x10^9/L in either granulocyte concentration was observed. However, the median (range) time to platelet >50x10^9/L containing >10% granulocytes was 27.2 (12-87), and that for cells containing <10% granulocyte was 20.3 (10-51), respectively. There was significant difference in the day to platelet >50x10^9/L between the two groups (p=0.04, respectively).

Summary/Conclusions: Long-term cryopreservation represents a means of holding a potential therapeutic modality in reserve for use at a future date. In this study, PBSCs can safely be stored for at least 36 months with 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. Difference in the day to plateau 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.

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 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 hematopoiesis, and the parameters of the infused product (CD34+ cells and LTx10^8/kg r), type of transplant, GVHD presentation, treatment and reactivation of CMV on the recovery of absolute lymphocyte numbers in s+30 and +60 days post transplantation using as cut off <3x10^9/mL. We have analyzed the ratio of the number of lymphocytes on day +60 with survival after transplantation. It has made a statistical - analysis of OS and DFS in relation to the number of lymphocytes or day +30 and day +60 with Kaplan Meier compared the results with long-rank test and subsequent analysis of the variables collected with Cox Regression.

Results: After analyzing the product infused we observe a relationship between LT and lymphocyte recovery on day+30 (p=0.097, cor: 0.223) and day +60 (p=0.059, cor: 0.257) but not with the CD34+Kg + X. Table 2 shows the patient characteristics in lymphocyte absolute count in the day +60. We analyzed the overall survival (OS) and disease - free survival (DFS) and a decrease in OS with statistical difference was evident in patients with <300 (p=0.0029) on day +60 and day+30 (p: 0.05), a decline also in DFS, with no statistically significant difference (p=0.1). Multivariate analysis to determine which factors could influence the lymphoid recovery on day +60 and SG, we observed that the type of unrelated donor, myeloablative conditioning and ATG administration can influence a delay in a recovery. No differences were observed in the rest of the variables.

Table 1.

Summary/Conclusions: A delay in lymphocyte recovery is associated with a decrease in survival rates in our patients. Measures favoring an accelerated lymphocyte recovery (prudent use of thymoglobulin, adequate donor selection, and transplantation modality) could affect the post-transplant survival. It appears that the amount of infused product could play an important role in reconstitution, so it would be a factor to take into account prior to infusion.
PB2182
SUCCESFULL AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER VELCADE-BASED REFRACTIVE MULTIPLE MYELOMA PATIENTS

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Background: The optimal induction treatment for Newly Diagnosed Multiple Myeloma Patients needs combinations with Bortezomib-Based (Bor-based) schemes. Primary Refractory patients include patients with progressive disease or rapid (<60 d) relapse after these optimal induction approach have a very bad prognosis. Lenalidomide-Dexamethasone (LenDex) were usually the next step in the treatment of these patients, until the recent introduction of triplets combination LenDex-based. Autologous Stem Cell Transplantation (ASCT) have shown efficacy in NDMM younger patients that have got at least a partial response (PR) after the induction therapy. There are few data about toxicity and response of ASCT in primary refractory patient that can obtain a response with LenDex rescue treatment.

Aims: Analysis of tolerance, response and overall survival of ASCT-candidates that are primary refractory to Bor-Based induction treatment.

Methods: Retrospective analysis of our database. From 2010 to Nov-2016, 53 ASCT-Candidates (for 1st or 2nd ASCT procedures) were included. Median Age for diagnosis was 62 (46-71). Median Age for ASCT procedure was 63 (46-72). 12 of these 53 patients (22.6%) were considered primary refractory and considered candidates to get 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 I/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 (2) and disease progression at diagnosis (3) of the patients. Median number of cycles administered: 6 (3-12). Of them didn’t respond. Of the other 9, 6 of them were considered candidate to intensificate treatment with high doses chemotherapy supported with an ASCT (2 of 6 to a 2nd ASCT procedure). The other 3 patients are in treatment or in preASCT evaluation. Characteristics of the after-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 conversion of refractory to partial response. New combinations (triplet) 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 (RTC) regimen to decrease relapse rate after allogeneic stem cell transplant (allo-SCT) without increasing non-relapse mortality (NRM), has not been well established.

Aims: In this retrospective study at the American University of Beirut medical center (AUBMC) we aimed to evaluate the outcomes of patients who underwent allo-SCT with thiotepa, busulfan and fludarabine (TBF) as RTC.

Methods: We included twenty four consecutive patients with hematological malignancies who received TBF as conditioning for allo-SCT from January to December 2016. All patients and transplant characteristics are listed in Table 1. All patients received the myeloablative conditioning regimen consisting of thiotepa(5mg/kg/day) infused on day -7 and -6, fludarabine(30mg/m2/day) was infused on day -5 to day -2, and busulfan(130mg/m2/day) was infused on day -5 to day -3. All patients received 2.5mg/kg/day intravenous rabbit antihuman monoclonal antibody (ATG) on days -2 and -1. GVHD prophylaxis for patients transplanted from haploidentical donor consisted of post-transplant cyclophosphamide 50mg/kg/day on day +3 and +5, cyclosporine started at 3 mg/kg/day on day +4 and readjusted according to level, and mycophenolate mofetil 500mg/4day started on day +6 to +28. Patients transplanted from matched related donor, received cyclosporine as of day +1.

Results: Twenty three patients(96%) engrafted, with 14 days (range, 10-18) neutrophil engraftment respectively. One patient who underwent haploidentical donor transplant with persistent disease for AML (karyotype 45,XY,-7) failed to engraft and died due to disease progression on day+22. After a median follow up of 10 months (range, 1-22) post-allo-SCT, the cumulative incidence of Grade II-IV acute GVHD (aGVHD) was 26%. One patient developed chronic limited GVHD (cGVHD). All the complication post allo-SCT are listed in table 1. Five patients (24%) relapsed post allo-SCT at a median of 163 days (range, 55-384), of whom 3 (60%) due to disease progression and two were successfully salvaged and are in complete remission (CR) with full donor chimerism (FDC) at last follow up. Two patients developed JC virus progressive multifocal leukoencephalopathy, one of them made a full recovery and the other died in CR. The day 100 NRM was 0%. At last follow up 20 patients (83%) are alive in CR, with negative minimal residual disease and FDC.

Table 1.

Summary/Conclusions: Our results show that This TBF conditioning regimen appears to be safe, allows high rate of engraftment and low NRM rate among high-risk patients and can lead to a long-term disease control.

PB2184
COMPLETE REMISSION STATUS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION AS PROGNOSTIC FACTOR IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is commonly used for treatment of relapsed or refractory non-Hodgkin’s lymphoma (NHL), as well as for first-remission consolidation in patients with mantle cell lymphoma. Disease status before ASCT is variable and is unclear whether complete response before ASCT or after ASCT correlates with better survival.

Aims: To evaluate the prognostic effect of disease status before ASCT - complete remission (CR) vs partial remission (PR) - in a cohort of patients with NHL.

Methods: Retrospective analysis of patients with NHL treated with HDC and ASCT between 2007 and 2017 in a single institution. All patients received peripheral blood stem cell support after conditioning with BEAM regimen (carmustine 300mg/m2, etoposide 800mg/m2, Ara-c 1600mg/m2 and melphalan140 mg/m2). Response was assessed according to The Lugano Classification. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between risk groups was performed by using the log-rank test (1%).

Results: 53 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 (15.7%). 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 (0.3-117.6), OS at 2 and 5 years was 84.8%
and 74.5% and PFS was 76.8% and 58.2%, respectively. Before ASCT, 60 patients (72.3%) were in CR and 23 (27.7%) were in PR. After 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 predicted CR (HR 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 MANTEL 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 male NHL patients. The patients were followed after ASCT for relapse.

Results: Patients were followed by a median time of 56.9 months (range, 6-170 months). The median age at diagnosis was 45 (range, 18-69), female to male ratio=5/16. The stages and MIPI scores at diagnoses were as follows: 5% stage I, 19% stage III, 76% stage IV; Low MIPI 29%, intermediate MIPI 48% and high MIPI 23%. First line treatments were R-CHOP for 6 cycles in 4 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, 4-123 months). Ten patients received BORID (bortezomib, rituximab, dexamethasone) and 1 patient received lenalidomide as salvage therapy and six of them achieved complete remission. Three patients underwent autologous hematopoietic stem cell transplantation as well as two patients received autologous peripheral blood stem cell transplantation followed by chemotherapy.

Summary/Conclusions: ASCT is a part of initial treatment strategy in fit patients with mantle cell lymphoma however 19 patients in our series had transplant related toxicity. Today, novel agents may present a less intensive approach for achieving response.

PB2186

ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH AUTISM

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Background: Autism Spectrum Disorders (ASD) are severe heterogeneous neurodevelopmental abnormalities characterized by dysfunctions in social interaction and communication skills, restricted interests, repetitive and stereotypic and non-verbal behaviors. The etiology of ASD remains unknown, but recent studies suggest a possible association with altered immune responses and ASD. Inflammation in the brain and Central Nervous System has been reported with microglia activation and increased cytokine production in post-mortem brain specimens of individuals with ASD. Other studies have established a correlation between ASD and family history of autoimmune diseases, associations with MHC complex haplotypes, and abnormal levels of various inflammatory cytokines and immunological markers in the blood. The 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 behaviors.

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 behaviors. 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 30 (Mild-to-Moderate Symptoms of ASD), 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 cord blood autologous stem cell transplantation as well as two patients received autologous peripheral blood stem cell transplantation followed by chemotherapy.

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 4.3 years and disease duration from diagnosis to transplantation ranged from 3 to 36 months with a median of 10 months. Five patients had primary and one had secondary MDS. Four patients had juvenile myelomonocytic leukemia (JMLM) and 4 patients had myelodysplastic related acute myeloid leukemia (MDR-AML). Diagnostic cytogenetics included monosomy 7 (n=2), trisomy 8 (n=3), KRAS (n=1) or normal/other (n=8). Patients received a median of 6.8x109 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), 5 from a matched unrelated donor (MUD) and 4 from haploidentical donor. Conditioning regimen consisted of busulfan/cyclophosphamide in MSD/MUD patients. The patients transplanted from MUD and UCB also received antithymocyte globulin (ATG) for 3–5 days pretransplantation. Haploidentical transplantation was performed with RIC regimen and TCRα/CD3 depletion.

Results: Graft failure occurred in three patients with JMLM (n=1), secondary MDS (n=1) and MDR-AML (n=1). Except one, all of them were transplanted at a median 83.5 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 behave like low-risk. Four patients died from transplant-related toxicity (n=2) and relapse (n=2). For the entire group, estimated five-year relapse-free survival (RFS), event-free survival (EFS) and overall survival (OS) were 78.6%, 64.3% and 70.7%, respectively.

Summary/Conclusions: These data demonstrate that especially children with primary MDS may achieve encouraging OS and RFS following HSCT. Relapse remains the main cause of treatment failure in children with JMLM given HSCT. All children with MDS should be referred for allogeneic HSCT soon after diagnosis.
PB2188

RELATIONSHIP BETWEEN URIC ACID LEVELS AND CARDIAL FINDINGS IN A LARGE COHORT OF Β-THALASSEMA MAJOR: GENDER-RELATED DIFFERENCES

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1Fondazione G. Monasterio CNR-Regione Toscana, Pisa, 2Azienda Ospedaliera Regionale San Carlo di Potenza, Potenza, 3Azienda Ospedaliera Cosenza - Presidio Ospedaliero “Annunziata”, Cosenza, 4Modena, Policlinico di Modena, 5Policlinico Umberto 1, Roma, 6Ospedale Casa Sollievo della Sofferenza IRCOS, San Giovanni Rotondo, Italy

Background: Iron overload, secondary to recurrent transfusions and ineffective erythropoesis, induces oxidative stress in thalassemia (TM). Uric acid (UA), a major blood antioxidant, may act either as an antioxidant or pro-oxidant.

Aims: Our aim was to evaluate the role of UA in TM and its association with cardiac iron, dysfunction, fibrosis, and complications, and cardiovascular risk factors in a large cohort of TM patients of both sexes.

Methods: 397 TM patients (200 men, mean age 32±8 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network were considered. Myocardial alterations in this study were quantified by the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: As expected, UA resulted significantly higher in male respect to female TM patients (4.7±4.1 vs 4.0±1.0 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±0.9 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 (P=0.01), and BMI (P=0.05) remained as independent determinants of UA in male TM patients.

Summary/Conclusions: UA levels correlate with factors related to metabolic dysfunction in TM patients of both sex, while a more strong correlation between UA and cardiac fibrosis was observed only in females, and a direct relationship between UA and T2* global heart only in males. The differences in male and female TM patients imply some gender-specific mechanisms, providing biochemical basis for the epidemiological differences between sexes.

PB2189

CHARACTERIZATION OF HEMORHEOLOGICAL ALTERATIONS IN THALASSEMA BY A CHEMOMETRIC APPROACH

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Background: Several studies reported a high incidence of thromboembolic events in β-thalassemia, more frequent in thalassemia intermedia than in regularly transfused thalassemia major. In these patients a chronic hypercoagulable state is evident and the red blood cells exhibit impaired flow properties that facilitate micro-circulatory disorders.

Aims: Since many abnormalities described in thalassemia may determine rheological alterations, 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 the patient status.

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 a clear identification of the rheological profile of thalassemia patients. Increased G’, G’’ and G’ modula were observed in thalassemia 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 THALASSEMA 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, transmitted 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 in transfusion-dependent Thalassemia patients, followed up in a single Thalassemia Unit, in Northern Greece.

Methods: Over a one-month period, we retrospectively evaluated 96 consecutive patients, from a registry of 150 adult TDT patients followed at a single Thalassemia Unit, in Northern Greece. The mean age of the study population was 22 years (30% were <10 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@ceeerTools 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 implied that there was no history of previous HE infection. According to the literature, IgG antibodies may be detectable following an HE infection for a time period that varies from one year to 14 years.

Summary/Conclusions: This is the first assessment of the HE virus seroprevalence in the population of TDT patients in Greece, over the last two decades. Our results suggest that TDT patients are not at a high risk for HE infection. Further studies are necessary to evaluate the clinical importance of the transfusion-transmitted HE infection in TDT patients and clarify whether screening of blood donors is necessary for countries with a lower or higher prevalence of HE.

PB2191

Abstract withdrawn.

PB2192

A PRELIMINARY STUDY OF THE CARDIAC EFFECT OF PPAR GAMMA IN BETA THALASSEMA MAJOR WITH IRON OVERLOAD

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Background: Peroxisome proliferator–activated receptor (PPAR)-gamma is a transcription factor belonging to the same family of nuclear receptors as steroid and thyroid hormone receptors. PPAR-gamma is a master transcriptional regulator involved in the expression of probably hundreds of genes. One of PPAR gamma gene polymorphisms is Pro12Ala which is present in at least 80% of normal populations. Pro12Ala polymorphism may reduce the risk of cardiovascular complications. Consistently, Ala12 allele carriers were found to have lower carotid intima-media thickness and reduced risk of myocardial infarction in type 2 diabetes patients. Pharmacological agonists of PPAR-gamma leads to a molecular
switch providing alleviating myocardial injury through modulating oxidative, inflammatory and apoptotic signaling pathway.

Aims: Our aim was to investigate the frequency of Pro12Ala polymorphism (substitution of proline to alanine at codon 12 in exon B of PPARγ gene in Egyptian β-thalassemia major (β-TM) with iron overload. Untreated transfusion induced iron overload in thalassemia major is fatal, usually as a result of cardiac complications.

Methods: 30 β-TM patients and 10 healthy volunteer matched for age, sex and body weight were involved in this study. β-TM patients followed up was in the “outpatient clinic of Hematology unit, at Alexandria main university hospital”. Seventeen were males and thirteen were females with ages ranging from 16 – 39 years (21.5±4.44). Blood samples from β-TM patients and healthy controls were analyzed for PPARγ gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism.

Results: The mean value of serum ferritin in β-TM was 4976.30±2216.41 ng/L which was significantly higher than that in controls (102.60±12.69 ng/L). The mean value of ejection fraction were 62.23±3.46% and 63.80±4.34 in cases and controls respectively. Pro12Ala polymorphism was present in 2 out of 30 (6.67%) β-TM patients with osteoporosis. One patient had heterozygous 12Ala polymorphism and the other had homozygous 12Ala polymorphism. Both had normal body mass index, lipid profile, ejection fraction and elevated serum ferritin (4923 ng/l in heterozygous patient and 4886 ng/l in 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.

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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

THALASSEMAIA MAJOR AND INTERMEDIA IN PATIENTS OLDER THAN THIRTY-FIVE YEARS - FROM A FATAL TO A CHRONIC DISEASE

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Background: During the past four decades beta thalassemia major (TM) and beta thalassemia intermedia (TI) have transformed from a universally fatal disease 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, further treatment options are still needed to confirm this finding.

Methods: The mean value of serum ferritin in β-TM was 4976.30±2216.41 ng/L which was significantly higher than that in controls (102.60±12.69 ng/L). The mean value of ejection fraction were 62.23±3.46% and 63.80±4.34 in cases and controls respectively. Pro12Ala polymorphism was present in 2 out of 30 (6.67%) β-TM patients with osteoporosis. One patient had heterozygous 12Ala polymorphism and the other had homozygous 12Ala polymorphism. Both had normal body mass index, lipid profile, ejection fraction and elevated serum ferritin (4923 ng/l in heterozygous patient and 4886 ng/l in 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).

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<td>60.80±2.67</td>
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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.

PB2194

EVALUATION OF LIVER IRON CONCENTRATIONS IN CHILDREN WITH BETA THALASSEMIA INFECTED WITH HEPATITIS C VIRUS BEFORE AND AFTER SPIRULINA THERAPY

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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.

Methods: Thirty multi-transfused β-thalassemic 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...
(group 1) and moderate to severe group (group 2). In addition to standard packed red cell transfusion, Spirulina therapy was given orally for 3 months, after which re-evaluation of these children was performed by repeating the same investigations.

**Results:** There was significant increase in LIC associated with significant changes in other MRI parameters (significant decrease in T2* and significant increase in R2*) in patients with β-Thalassemia of moderate to severe group as compared to those of the mild group before treatment. The mean values of serum ferritin (SF) was statistically insignificantly higher among patients of mild group. There was no significant correlation between different MRI parameters and SF level. There was negative correlations between LIC and T2* and positive correlation between LIC and R2*. There was significant decrease in values of LIC accompanied with significant improvements in SF after spirulina therapy as compared to their pretreatment values in patients of the moderate to severe group.

**Figure 1.**

**Summary/Conclusions:** Spirulina therapy may have favorable effects on lowering the values of LIC in children with β-Thalassemia infected with HCV.

**PB2195**

**COMBINATION OF DEFERASIROX AND DEFEROXAMINE - A SUCCESSFUL CHELATION THERAPY IN B-TALASSEMA MAJOR PATIENTS**

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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 thalassemia (TDT) patients attending the Thalassemia Unit in a tertiary hospital in Athens, Greece.

**Methods:** 10 TDT patients were treated with a combination chelation therapy of DFX (30 ±10 mg/kg/d) and DFO (44±12 mg/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) monotherapy is not effective or not well tolerated.

**Results:** Five of the 10 patients had significant liver hemosiderosis (LIC >15 mg Fe/g liver d.w.) and 3 had heart iron overload, of which one significant (T2*1.9 msec). There was significant decrease in values of LIC accompanied with significant improvements in SF after spirulina therapy as compared to their pretreatment values in patients of the moderate to severe group.

**Summary/Conclusions:** Spirulina therapy may have favorable effects on lowering the values of LIC in children with β-Thalassemia infected with HCV.

**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 separation devices for their capacity of performing the diagnostics of haemoglobinopathies. These involve the Variant II TM HPLC (BioRad), the Capillaries2 capillary electrophoresis (Sebia) and the most recently introduced HPLC, Premier Hb9210TM High Resolution HPLC of Trinity Biotech (Menarini).

**Aims:** As the latter device is new to the market a multisite precision study was performed testing the reproducibility of the system across three test sites (Leiden, Genoa and London) using the same set of samples for several following days. The results between these three sites were compared and evaluated. Moreover we have tested the capacity to detect the most common structural haemoglobin variants, such as HbS, HbC, HbE and less common Hb variants important to be diagnosed in multi-ethnic populations found in the U.K., the Netherlands and Northern Italy as well as elevated HbA2, as indicator for beta-thalassaemia carriers.

**Methods:** Hb variant separation using he Variant II TM HPLC (BioRad), the Capillaries2 capillary electrophoresis (Sebia) and the most recently introduced HPLC, Premier Hb9210TM High Resolution HPLC of Trinity Biotech (Menarini).

**Results:** We present the data of the comparison studies using the replicates of the three different sites for the Premier Hb9210TM and of 100 normal samples and 217 patient samples for a variety of beta-thalassaemia trait and haemoglobin (Hb) variants, including the molecular data of the beta-thalassaemia mutations and Hb variants.

**Summary/Conclusions:** All three apparatus identified the common Hb variants and beta-thalassaemia trait in carriers, homo- hetero- and compound heterozygotes with the expected sensitivity and specificity. The Premier Hb9210TM HPLC shows comparable separation of Hb variants determined by quantitation on the three different sites using the same samples and is suitable for the analysis of samples suspected of having haemoglobinopathy and the diagnosis of beta-thalassaemia trait or Hb variants.

**PB2197**

**RED BLOOD CELL EXTENDED PARAMETERS IN HAEMOGLOBINOPATHIES**

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**Background:** Sysmex® XE-5000 analyzer incorporates new research Red Blood Cell (RBC) parameters, derived from flow fluorescence cytometry technology, including%HYPO-H, which indicates the percentage of RBC with haemoglobin (Hb) content <17 pg, and%MicroR which indicates the percentage of RBC with mean cell volume <60 fl.

**Aims:** The aim of this study was to establish the reference range of our Laboratory for the parameters%HYPO-H and%MicroR, to investigate their values in haemoglobinopathies and their correlation, if any, with Hba1c levels in heterozygous β-thalassaemia.

**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, 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-H &%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±SD 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-H &%MicroR are 0.0 – 0.6% & 0.2 – 2.9%, respectively, and they are independent of gender and age (P=0.715, P=0.168 & P=0.073, P=0.843). There was no statistically significant difference between the groups determined by one-way ANOVA for both parameters (all P >0.0001). Heterozygous β-thalassemia presents statistically significantly higher%HYPO-H values [11.6 (2.4-27.6)] as compared to groups A [0.3 (0.2-0.3)], C [1.9 (0.6-4.6)], D [0.6 (0.4-0.8)] (all P <0.0001), while there was no statistically significant difference of%HYPO-H values between heterozygous Hb O-Arab and groups A and C (P=0.965 & P=0.134, respectively) based on Tukey post hoc test. 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
The average number of transfusion in last 5 years. Majority of patients (56%) were male. The average age at diagnosis and blood transfusions were included in the study.

Methods: In this prospective observational study we analysed demographic details, clinical features and cardiac and liver iron assessment in these chronically transfused individuals. Multiplex ligation probe amplification® (MLPA®) is a molecular diagnostic 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 - c.3,77, heterozygous - c.4.2 (g.285) 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® for comparison. A comparison of diagnostic performance, interpretation, turnaround time (TAT) and costs (reagent and labour) was conducted.

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. 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 years. 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 T2*MRI finding at the time of registration was 2919 mg/kg.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 was 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, unicentric, 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 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. Qutelation 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 stem cell transplantation and three 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.

<table>
<thead>
<tr>
<th>Not sickle/</th>
<th>Hemoglobinopathies</th>
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<tr>
<td>Homologous</td>
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<td>(C, D, Andrew-Minniearl, J Baltimore)</td>
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<tr>
<td>Hb CC</td>
<td>4 (20.04)</td>
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<td>Hb DC</td>
<td>2 (9.96)</td>
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<td>3 (15.03)</td>
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<tr>
<td>Hb H</td>
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<tr>
<td>Hb HbO-Arab</td>
<td>2 (9.96)</td>
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<tr>
<td>Hb HS</td>
<td>2 (9.96)</td>
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**Summary/Conclusions:** Early diagnosis derived from universal neonatal screening for sickle cell disease allows an effective health education and prompt therapy to other hemoglobinopathies, and a correct and thorough follow-up of these patients.

**PB2201**

**PREVALENCE AND CAUSES OF CLOTTING TIMES PROLONGATION IN PATIENTS WITH TRANSFUSION DEPENDENT BETA THALASSEMIA**

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**Background:** Thalassemia is traditionally known to be a thrombophilic, rather than hemorrhagic, disorder. In spite of this, prolongation of clotting times are often reported. Understanding if there is a real risk of bleeding, and what this risk can be associated to, is crucial, especially in relation to the frequent referral to surgery (e.g. for splenectomy, cholecystectomy). Hepatopathy due to iron overload or HCV infection has been addressed as a main cause of this finding, even though disorders in the clotting profile are often reported also in patients with no alterations of hepatic function. The impairment of factors XI and XII often reported has been hypothesized to be secondary to intravascular haemolysis or multiple transfusions (Casco et al, Acta Haema- tol 1978, Mifadyen 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 Transfu- sion dependent Thalassemia (TDT) patients and to assess the correlation with hepatopathy, degree of the hemolysis, transfusion frequency, erythroblastosis, iron chelation.

**Methods:** TDT patients followed at our center for whom clotting tests were available were included. From chart review data were collected regarding clotting times, demographics, disease history, comorbidities and concomitant medica- tions, iron chelation therapies, iron overload (serum ferritin, LIC, cardiac T2*), liver function tests, hemolysis parameters, hemocromocitometric values. Patients on anticoagulation therapy were excluded.

**Results:** 20 TDT patients (female 55.35%) were enrolled in our study, mean age 26.02±13.38 years, 17 of them were pediatric. In 20/56 patients (35.71%) prolongation of clotting time was found: this included both prolonged INR (23.21%) and prolonged aPTT ratio (25%); 7 patients (12.5%) had both prolonged INR and aPTT. Subgroup with clotting disorder (group A) was compared to subgroup with clotting times within normal ranges (group B) using T-Test. No differences were found in terms of sex, age, genotye, transfusion interval, hemolysis degree, comorbidities, HCV infection included, iron overload, liver function, erythroblastosis and platelets levels, nor in history of thrombotic complications. No patients had history of hemorrhagic disease. Pretransfusion Hb was lower in patients with prolonged clotting times (p=0.045); none of the patients in Group A was splenectomized (p=0.042).

**Summary/Conclusions:** In our population clotting disorders were not correlated with hepatic disease, nor hemolysis or transfusions. The mild corre- lation with lower Hb values and with the lacking splenectomy could be consistent with the known effect of low Hb on lab procedures for clotting tests. In relation to this observation in patients with altered coagulation tests the rep- etition of clotting test after blood transfusion could be advisable to overcome the low Hb effect.

**PB2202**

**COMPOUND HETEROZYGOSSITY FOR HAEMOGLOBIN ADANA AND A-THALASSAEMIA IN GREECE. CLINICAL PHENOTYPE AND GENETIC COUNSELING**

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**Background:** Haemoglobin (Hb) Adana (HBA2q.C.179>A) 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 a-thalassaemia 3.7 kb deletion - the only α- 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, RBC 4.04 X 10¹²/L, 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, RBC 5.88 X 10¹²/L, MCV 73.1 fl, MCH 23.5 pg, Hb A2 2.4% and Hb F1% and inclusion bodies were found positive. DNA analysis was requested and, routine investigation was performed in their first offspring. The girl had an Hb of 8.2 g/dl, RBC 3.82 X 10¹²/L, MCV 70 fl, MCH 22 pg, Hb A2 1.9% 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 mother carried the α-thalassaemia 3.7 kb deletion defect. The father carried the rare non deletional Hb Adana. As suspected from the haematologi- cal data, their offspring was a compound heterozygote for Hb Adana variant in trans to a 3.7 α+ thal deletion. The second case concerns a 17 year-old boy, diagnosed with Hb Adana co-inheritance with the a-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 10.9g/dl, RBC 4.77 X 10¹²/L, MCH 23 pg, RDW 18.6%, reticulo- cyte 5%), until age of 11 when 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, neces- sitating chelation therapy. Weight, height and pubertal development were nor- mal 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 pheno- type. However, the co-inheritance of Hb Adana with the 3.7 kb α+ thal deletion is rare, only with 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 demon- strated 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 manage- ment to the patients and the most accurate genetic counseling.
Thrombosis and vascular biology

PB2203

ANTITHROMBOTIC EFFECTS OF PEPTIDE PGPL IN EXPERIMENTAL THROMBUS FORMATION

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Background: Previously, it was established that proline- and glycine-containing peptides have fibrinolytic, anticoagulant activity, inhibit platelet aggregation and thrombin activity in vitro and in vivo. Besides, it is known that short peptides of this family also have antithrombotic effects.

Aims: To study the influence of Pro-Gly-Pro-Leu (PGPL) and amino-acid leucine on fibrinolytic and anticoagulant blood activity, platelet aggregation and to estimate their possibility to reduce the formation of experimental blood clots.

Methods: Experiments were carried out on white rats (200–250 g) according to the ethical principles of the Helsinki Declaration. Peptide PGPL (1 mg/kg), leucine (0.33 mg/kg - equivalent to its content in PGPL) and saline (control rats) were intranasal entered to rats within 3 days. 1 hour after the last drugs administration we induced the formation of thrombus in n=4 juuginals (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 of 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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1Department of Haematology in Ampang Hospital, Ministry of Health, Selangor, Malaysia

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, Inflammatory TMA, SLE related TMA 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 laboratory 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.

Background: Previously, it was established that proline- and glycine-containing peptides have fibrinolytic, anticoagulant activity, inhibit platelet aggregation and thrombin activity in vitro and in vivo. Besides, it is known that short peptides of this family also have antithrombotic effects.

Aims: To study the influence of Pro-Gly-Pro-Leu (PGPL) and amino-acid leucine on fibrinolytic and anticoagulant blood activity, platelet aggregation and to estimate their possibility to reduce the formation of experimental blood clots.

Methods: Experiments were carried out on white rats (200–250 g) according to the ethical principles of the Helsinki Declaration. Peptide PGPL (1 mg/kg), leucine (0.33 mg/kg - equivalent to its content in PGPL) and saline (control rats) were intranasal entered to rats within 3 days. 1 hour after the last drugs administration we induced the formation of thrombus in n=4 juuginals (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.

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Summary/Conclusions: Thus administration of PGPL enhanced of 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.

PB2205

ANALYSIS OF ADAMTS-13 ACTIVITY AND RELAPSE RISK IN PATIENTS WITH PRIMARY ANTITHROMBOTIC DEFICIENCY

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1Biology, Lomonosov Moscow State University, Moscow, Russian Federation

Background: ADAMTS-13 activity testing. There were 24% Primary Acquired TTP, 5% typical HUS, 6% atypical HUS, 16% Drug induced TMA, 21% Malignancy associated TMA, 31% SLE related TMA and 12% Transplant TMA.

Aims: To study the influence of Pro-Gly-Pro-Leu (PGPL) and amino-acid leucine on fibrinolytic and anticoagulant blood activity, platelet aggregation and to estimate their possibility to reduce the formation of experimental blood clots.

Methods: Experiments were carried out on white rats (200–250 g) according to the ethical principles of the Helsinki Declaration. Peptide PGPL (1 mg/kg), leucine (0.33 mg/kg - equivalent to its content in PGPL) and saline (control rats) were intranasal entered to rats within 3 days. 1 hour after the last drugs administration we induced the formation of thrombus in n=4 juuginals (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 of 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.

PB2206

PREVALENCE OF ANTI PHOSPHOLIPID ANTIBODY AND HBA1C IN T2DM WITH DIABETIC VASCULAR COMPLICATIONS

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Background: Anti-phospholipid antibodies (APA) are a group of proteins directed against the phospholipids of cell membranes, such as cardiolipins or phospholipid binding proteins. APA presence provokes microvascular, arterial or venous thrombotic events indicating somehow the relationship between the immune system, the hemostatic system, and the inflammatory response. It has been suggested that their presence in a critically ill patient is related to thrombotic manifestations, organ dysfunction, and death.

Aims: The aim of this study was to evaluate the prevalence of anti phospholipid antibodies in critically ill patients with autoimmune diseases and the rate of organ involvement.

Methods: Retrospective and descriptive study of patients admitted to the intensive care unit of Hospital Universitario de la Samaritana between 2008 and 2016, in Bogotá, Colombia.

Results: A total of 79 patients were found to have systemic lupus erythematosus (SLE), antiphospholipid syndrome and vasculitis. 17 patients (22%) were positive for antiphospholipid antibodies. Of these, 76% were women and mean age was 38 years (18-63 years). APA profiles showed positivity with this distribution: one positive antibody, n=9 patients (53%) (lupus anticoagulant antibody being the most common), two positive antibodies in n=4 patients (23%) and three positive antibodies in n=4 patients. Anemia (100%), monocytosis (64%), thrombocytopenia (40%) and prolonged INR (17%) were found in 88% of patients on admission to the ICU. In descending order, other organ involvement was found to be: pulmonary and renal dysfunction (70%), shock (53%), central nervous system involvement (41%), cardiovascular (23%), and gastrointestinal (22%). 82% of this cohort had positive anti-nuclear antibodies (ANA) and 23% anti-cytoplasmic antibodies (ANCA). 100% of patients had elevated C-reactive protein (CRP), and APACHE II score average was 11 points (Table 1).

Table 1.

Summary/Conclusions: Hematologic, renal and pulmonary involvement are the most commonly compromised in patients with antiphospholipid antibodies positivity in patients with autoimmune diseases in the ICU. Based on these results, a prospective study is proposed in order to evaluate the presence of APA and their impact on mortality and multi-organ dysfunction in these patients.
Background: Antiphospholipid antibodies (APLS) have been implicated in cardiovascular and thrombotic disorders. 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: (a) control subjects without T2DM, (b) uncomplicated T2DM and health control. Each had 70 subjects matched for sex and age. Lupus anticoagulant (LA) was assayed using DRVVT (technoclon GmbH Austria) IgG/B2GPI-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 control subjects respectively. ANOVA showed a significant difference in mean position on the risk of acute coronary syndrome (ACS). Post hoc analysis showed this difference was between complicated T2DM and healthy controls (p<0.001, 95%, CI:3-0.2 to -1.9) and in uncomplicated T2DM and healthy control subjects (p<0.001, 95%, CL:2.8 to -2.0) there was a significant difference in mean position on the risk of acute coronary syndrome (ACS). HbA1C 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.

Summary/Conclusions: The study did not find any causal or other association between T2DM and the occurrence of APLS positivity, however, APLS may be simply an aggravating factor for vascular complications especially in poor controlled T2DM.

PB2209

IMPORTANCE OF MONITORING PATIENTS WITH DIRECT ORAL ANTICOAGULANTS

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Background: A major advantage of these agents is the lack of a requirement for routine monitoring. However, some patients do present clinical conditions that may lead to renal dysfunction, drug-drug interactions, etc., however it’s recommended monitoring the drug for Rivaroxaban Apxaban and Endoxaban use anti-Xa chromogenic studies and for Dabigatran Hemoclot for the drug, however for dabigatran it’s recommended checking the PT and APTT and PT and Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured in CLOTimer analyzer. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured in CLOTimer analyzer. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured in CLOTimer analyzer. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured in CLOTimer analyzer. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured in CLOTimer analyzer.

Aims: To 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 Apxaban (26%). We analyzed the variables that increases the bleeding risk such as drug-drug interactions, renal dysfunction. Results: 10% of patients with Dabigatran developed bleeding of which 1.5% with Apixaban developed bleeding of which 1.5% with Apixaban developed bleeding of which 1.5% with Apixaban developed bleeding of which 1.5% with Apixaban developed bleeding of which 1.5% with Apixaban developed bleeding of which 1.5% with Apixaban developed bleeding of which 1.5% with Apixaban. A retrospective case-matched analysis was performed comparing 35 patients who developed bleeding with an equal number of patients who did, case and control groups were matched according to age, weight and measurement of serum creatinine we didn’t found significant difference.

Summary/Conclusions: We found that 6% of patients with Dabigatran, 2.5% with Rivaroxaban and 1.5% with Apixaban developed thrombotic episodes. Twenty percent of patient didn’t have therapeutic range of the drug. For each DOACs is shown in Table 1. We analyzed the patients who had hemorhage we found that all patients with Dabigatran prolonged the PT and APTT that the ACT in 80%, for other DOACs is shown in Table 1. A retrospective case-matched analysis was performed comparing 35 patients who developed bleeding with an equal number of patients who did, case and control groups were matched according to age, weight and measurement of serum creatinine we didn’t found significant difference.

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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, Abu, Akwa 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 (26.7%) who had between 0-5 years clinical experience. Least of the respondents (8.7%) had between 16-20 years clinical experience 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, 150 (28.4%) 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. 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 “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 which 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.

PB2214

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 cytokines. One of the important anti-inflammatory cytokine is interleukin-10 (IL-10) with important immunoregulatory functions. Primarily, IL-10 counterbalances the potentially harmful effects of tumor necrosis factor α (TNFα) and other pro-inflammatory mediator such as IL-1, IL-6, and IL-8 from monocytes/macrophages. Three important single nucleotide polymorphisms (SNP) have been identified in the IL-10 expression, including: 1082 A/G, 819 C/T, and 592 C/A. 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: A statistical analysis revealed that mutant genotypes distribution is statistically significant different compared to the wild genotype distribution, being higher in group A (with unprovoked DVT) than in group B (with provoked DVT); as GG genotype was detected in 14 patients (63.6%) versus 8 patients (36.4%) in group A and B respectively (P value=0.037); AG genotype was detected in 10 patients (43.5%) and 7 patients (30.4%) in group A and B respectively (P value=0.007). However, there is no correlation was found between IL101082 mutant genotype distributions and VTE recurrence (P value= 0.738 and 1 respectively) or positive family history of VTE (P value= 0.101 and 0.714 respectively), compared to wild genotype. IL10592AC genotype distribution of mutant genotypes (GG and AG) distribution showed no statistically significant difference (P value= 0.43 and 0.687 for GG and AG genotypes respectively) compared to wild genotypes distribution, also there is no correlation was found between IL10592AC mutant genotype distributions and VTE recurrence (P value= 1 and 0.284 for GG and AG genotypes respectively) compared to positive history of VTE (P value= 0.67 and 1 for GG and AG genotypes respectively), compared to wild genotype (AA).

Summary/Conclusions: IL101082AG gene polymorphism is associated with risk of unprovoked DVT, however it is not associated with either risk of recurrence or positive family history.

**PB2215**

**CATASTROPHIC ANTI-PHOSPHOLIPID SYNDROME TRIGGERED BY SEPSIS. A PROSPECTIVE CASE STUDY HIGHLIGHTING BIOLOGICAL CONCEPTS AND MANAGEMENT STRATEGIES IN THIS COMPLEX AND LIFE THREATENING DISEASE**

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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. Catastrophic CAPS is associated with bleeding diathesis, 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: To study the effect of tripeptides Pro-Arg-Gly and Gly-Arg-Pro, containing arginine-containing peptides in healthy rats and rats with experimental MS.

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 episode was associated with respiratory dysfunction, 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: To study the effect of tripeptides Pro-Arg-Gly and Gly-Arg-Pro, containing arginine-containing peptides in healthy rats and rats with experimental MS.

Results: Among study sample, 134 patients had strokes and only 18 had TIA. The recurrence of stroke/TIA was observed in 13.2% of patients. The majority of patients (57.7%) have had either radiological evidence or thrombotic event. One fourth of patients had past thrombotic events while 12.5% had family history of thrombosis. Out of haematological correlates screened Lupus anticoagulant was the most common haematological correlate (n=16) and dysfibrinogenemia(n=11) had the next high prevalence. One patient was diagnosed with Essential thrombocythaemia and one with SLE. None of the patients were positive for screening tests done for sickle cell disease and PNH.

Summary/Conclusions: The haematological correlates were identified in 19% of our study sample. Among stroke profile only presence of past thrombotic episode was statistically significant and was associated with haematological disorders (P = 0.04). Therefore haematological disorders appear to be an important factor in etiological work up of stroke patients particularly in patients with past thrombotic events.

**PB2217**

**ANTIPLATELET AND FIBRINOLYTIC EFFECTS OF ARGinine-CONTAINING PEPTIDES IN HEALTHY RATS AND RATS WITH METABOLIC SYNDROME**

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Background: Currently, the number of diabetes, hypercholesterolemia, metabolic syndrome (MS) patients has increased sharply in the world. MS is metabolic disorders with increase of cholesterol and glucose levels, dyslipemia, endothelial dysfunction. This is accompanied by an increase in blood clotting, including platelet aggregation strengthening and reducing the activity of the plasminogen activator. Thus, the MS may predispose to venous thrombosis. It is known that, regulatory oligopeptides involved in the conservation normal functional activity of coagulation, anticoagulation, insular systems of the organism, fat metabolism. It is also known that some amino acids, particularly arginine, improve rheological properties of blood and reduce platelet aggregation.

Methods: Studies were carried out on Wistar rats weighing 300-350 g in accordance with the ethical principles of the Helsinki Declaration. Two groups of animals were used: healthy rats and rats with experimental MS. Peptides were intranasal injected in doses of 1 mg / kg once daily for 5 days. 0.85% NaCl solution was injected to control rats in the same time frame. MS in rats was caused by a hyper-cholesterol fat-rich diet (FD) for 6 weeks. Blood samples were taken from the jugular vein 1 hour after the last drug administration. Activity of t-PA (fibrin plate method) and ADP-induced platelet aggregation (standard method) were measured in blood plasma.

Results: The intranasal administration of peptides Gly-Arg-Pro, and Pro-Arg-Gly to healthy animals resulted a reduction of platelet aggregation by 23% and 53% respectively and compared with intact rats. Both peptides induced enhancement t-PA activity of 2 or 3.5 times respectively. In rats with experimental MS these effects were preserved, besides, platelet aggregation was decreased by 27% (Pro-Arg-Gly) and 38% (Gly-Arg-Pro) compared with the control.
Summary/Conclusions: We concluded that intranasal administration of tripeptides Pro-Arg-Gly and Gly-Arg-Pro to organism of healthy rats and in rats with experimental MS show antplatelet 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 THROMBOMGRAM 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, Thrombinoscope compares the readings from the trigger wells and the calibrator wells, calculates thrombin concentration and displays the thrombin concentration in time. Outcomes from CAT were analyzed in the principal component analysis (PCA) which is a statistical procedure that allows us to summarize high dimensional data with a smaller number of representative variables that collectively explain most of the variability. Statistical analyses were performed with R 2.5.

Results: Four variables measured from CAT have different distribution and too large variations. For example, the mean(s.d) 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 variation 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 (s.d=0.583) (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (s.d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time</td>
<td>24.86(8.01)</td>
</tr>
<tr>
<td>Peak thrombin</td>
<td>80.16(94.52)</td>
</tr>
<tr>
<td>Time to peak</td>
<td>31.28(9.78)</td>
</tr>
<tr>
<td>Velocity index</td>
<td>19.08(28.86)</td>
</tr>
</tbody>
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Summary/Conclusions: The PCA value showed a good agreement with four CAT outcomes and less variation. The PCA method could be used to monitor the process of immunoglobulin manufacturing.

PB2219

PRIMARY THROMBOPHILIA IN MÉXICO XII: MISCARRIAGES ARE MORE FREQUENT IN PERSONS WITH THE STICKY PLATELET SYNDROME

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Background: Venous thromboembolism (VTE) comprising of deep vein thrombosis (DVT) and pulmonary embolism (PE) is one of the major cardiovascular causes of death along with MI and stroke. Though earlier works suggested that DVT is rarer in Asian population, recent studies have revealed that this might not be so. Most of the studies conducted in Asia in general and India specifically has been on hospitalized patients with minimal representation of young healthy individuals.

Aims: We aimed at studying the disease variables of VTE in young healthy males of Indian origin and compare the same with other Indian studies as well as the global statistics.

Methods: Hospital records of 176 Color Doppler Flow Index (CDFI) and/or Contrast Enhanced Computed Tomography (CECT) proven VTE patients being followed up in a tertiary care hospital was analyzed retrospectively to document cause (provoked/ unprovoked), venous systems involved, thrombophilia profile, duration of anti-coagulation and recurrence.

Results: Among the study population, 49.8% had a provoked VTE. 90.9% subjects had DVT, mostly of the lower limb. 15.3% had PE with DVT, 2.8% had PE alone and 6.2% had splanchnic vein thrombosis including portal vein thrombosis. In the subjects who had undergone thrombophilia profile, 41.9% had Protein C, 58.1% Protein S and 25.9% Antithrombin III deficiency. Lupus anticoagulant screen was positive for 13% of the screened subjects. The average duration of anti-coagulation was 16 months with majority (98.2%) patients being on Vitamin K antagonist. The recurrence rate in our study population was found to be 11.4% (Table 1).

Summary/Conclusions: Young Indian males have different disease variables
PB2221
A PRELIMINARY STUDY ON THE EFFECTS OF AMPHIBIAN CRUDE SKIN SECRETIONS ON SOME PARAMETERS OF HEMOSTATIC SYSTEM
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Background: A lot of bio-chemical compounds from secretion of the amphibian skin glands with various biological activities have been isolated and characterized. Several recent studies indicate that amphibian skin secretions can be a source of molecules affecting the platelet activity. We are interested to look for other bioactive components of the amphibian skin which exhibit ability to influence on diverse parameters of hemostatic system.

Aims: We performed a preliminary study of the some effects of amphibian skin secretions on hemostasis.

Methods: Adult specimens (both sexes) of Bombina bombina, Bombina variegata, Bufo bufo, and Bufoes viridis were collected from outdoors in Kyiv region of Ukraine. The crude skin secretions were collected by washing with ultrapure water and centrifuged to remove debris. The supernatants were 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, 2x10^5 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 37°C (Medtech, Russia). Coagulation parameters (prothrombin time (PT), thrombin time (TT), as well as activated partial thromboplastin time (APPT)) were measured by coagulometer (Rayto, RT-2201C) using corresponding commercial kits (Renam, Russia).

Results: The lyophilized B. bufo skin secretions in dose-dependent manner induced platelet aggregation in both PRP and purified PF; its final concentration of 50 mg of total protein/mL caused the same effect as 5x10^{-6}M ADP. These results indicated that skin components acted directly on platelets, maybe through their surface receptors. The lyophilized skin secretions of B. variegata and B. bufo also activated platelet aggregation but their effects were lower than B. bufo skin secretions. The skin secretions from all studied amphibian did not influence on PT and TT except B. viridis which prolonged TT by 40%. The values of APPT 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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22 nd Congress of the European Hematology Association

Background: Congenital plasminogen (Plg) deficiency is a rare autosomal recessive disorder that leads to the development of ligneous membranes on mucosal surfaces.

Aims: Here we report our experience with local and intravenous 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, ligneous membranes were surgically removed but all recurred. Nine patients were treated with intravenous 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 lost one eye before treatment. Two female patients and one male patient had undergone multiple surgeries for ligneous 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 completion of the pregnancy and the good health of the fetus.

Results: A total of 207 women were included into the study, 172 were treated with low molecular weight heparin and Aspirin while 35 were treated with just Aspirin. Out of 172 patients in the low molecular weight heparin group 155 managed to give birth which accounts for a 90% success rate with a reported case of fetal growth restriction and 2 cases of 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 preeclampsia was recorded. Out of the 35 women who did not receive treatment with low molecular weight heparin and only with Aspirin, 21 managed to complete their pregnancies representing the 60% out of which 2 cases presented with Abruption and 4 cases with fetal growth restriction, out of the 14 women who represented the 40% who were unsuccessful in completing their pregnancies 7 cases were recorded during the first trimester while 3 more had late fetal loss and 4 cases of preeclampsia.

Summary/Conclusions: Women treated for thrombophilia had a lower percentage of fetal loss than their no treatment group counterparts. There is an urgent need for appropriate guidelines for these patients in our medical center.

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 underdetermined (>2 weeks), minimum anticoagulation 3 months is recommended. If there are associated anatomical abnormalities, the possibility of surgical vascular thoracic decompression must be assessed.
Aims: To ascertain D-dimer diagnostic accuracy for upper extremity DVT.

Methods: A retrospective audit was undertaken to determine the aetiology and clinical presentation on patients who UDVT at presentations. Patients with a formal malignancy confirmed before the diagnosis was excluded. A D dimer (DD) with a cut off cut off levels validated for lower limb DVT was performed.

Results: A total of 18 patients were identified in the period of 2012 to 2016. All the cases investigations included Doppler US or CT/MRI and in 30% of the patients the thrombosis was confirmed via contrast venography as a reference standard test. The gender predominan was male in this group the symptomatology were related to physical efforts in a 60% (Paget-Shroëtter Syndrome) whereas in female serie the predominant was thrombophilic defects (factor V Leiden). The average age was 33 years (ranging from 21 to 68 years) and 2 elderly patients a new diagnosis of cancer was confirmed (thyroid and lung) (odds ratio, 3.24; 95% CI, 1.13-9.38). The 85% of the patients had an unprovoked event; four patients have a diagnosis of cathereter related thrombosis and four cases were noncathereter related thrombosis. Patients were treated in 60% (Paget-Shroëtter Syndrome) whereas in other serie the symptomatology were connected with the stimulation of the sympathetic nervous system. The patients had a diagnosis of SLE. We had four cases of positive DD screening (both were marginally elevated, P <0.01) . The risk of re-thrombosis was non significative but in the subanaylsis of relapsing thrombotic event populations the risk of relapse increased proportionally in relation of thrombophic defect and high BMI. A trend towards a higher rate of recurrent thrombosis (was observed among patients with BMI>25 (42.6%) compared to those with a BMI <25 (33%). This difference reached statistical significance in women with BMI>25, who had recurrent event in 51.7% of the cases vs those with BMI <25 (29.7%) (p <0.05 CI 0.03, 0.41).

Background: Many patients have an effect on the blood clotting system. It is known that there are heparin-like substances in some types of peony roots (Paeonia lactiflora, Paeonia suffruticosa). It proved that there is an anticoagulant activity in extracts from such roots.

Aims: The intention is to show the inhibitory effect of the extract of Paeonia lactiflora roots (EA) on processes fibrin and thrombus formation.

Methods: We used the standard coagulologic methods for determining anticoagulant activity by APTT test, antiplatelet, total fibrinolytic activity (TFA), fibrinopolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the human treatment of animals. We used an animal model with pronounced thrombogenic activity induced by administration of subthreshold doses of tissue thromboplastin at a dose of 0.6-0.7 ml per 200 g body weight in rats. After 30 min after injection of thromboplastin, we injected intraperitoneal-ly 0.1 mL of 1% of extract of EP and after 30 minutes we determined parameters of hemostasis in the blood plasma.

Results: It was shown that after administration of the indicated doses thromboplastin occurs hypercoagulability in blood plasma of animals (APTT decreased by 23% SFA - 15%, FDPA -12%; increased platelet aggregation by 18% compared to control animals not receiving thromboplastin). Normalization of blood clotting is installed in the experimental rats after application EP (recover y of platelet aggregation to 98%, APTT- to 100%, up to 95% SFA- FDPA and up to 67% compared with control). The high degree of FDPA indicates the ability of EP to obstruct the process of the formation of fibrins and thrombus.

Heparin components in EP interact with fibrin monomers which do not partici-pate in their conversion to fibrin polymer. As a result, stable fibrin polymer or thrombus is not formed.

Summary/Conclusions: Consequently, the extract of Paeonia lactiflora roots containing heparinoid contributes to the restoration of coagulation properties in blood of animals in prothrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.

Background: Rethrombosis and thromboembolia are the most common side effects of thrombolytic therapy. One of the possible causes of thrombosis is the entering of thromboplastin in the blood stream. Marker of thromboplastin is an intrinsic membrane glycoprotein 5'-nucleotidase (5'NT) that is present as an enzyme in a wide variety of cells. Recently it was shown that compensatory reaction of haemostasis system by using different fibrinolytic drugs was con-nected with the stimulation of the sympathetic nervous system. Besides, it is known that α–adrenoreceptor blockers have anticoagulant and antiplatelet effects. The prevention of thrombosis complication is very important field of pathophysiology and medical practices. Therefore, we studied effects of different α–adrenoreceptor antagonists and the influence of these substances combinations with various anticoagulant and fibrinolytic 5'NT were procured.

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-vention.

Methods: Experiments were carry out on 50 white laboratory rats weighing 200-230 g according to the ethical principles of the Helsinki Declaration. Anti-coagulant and antithrombotic effects of LMWH or HMWH were studied in two rat models of thrombosis – thrombosis in v. jugularis (Wessler) and thrombosis in arterio-venous shunt (direct registration of blood pressure). The α–AA digy-droagotexin (DET – 1mg/kg), α,α–AA azaprazin (PZ – 2mg/kg). LMWH or HMWH (40 USP/kg) were injected in v.jugularis. Saline was administrated in control rat groups. The thrombus were formed 15 or 180 min after substances injected. The degree of thrombus formation (TF) was detected in ball (Wessler model) and by time of TF (arterio-venous shunt model). In blood plasma the activity of 5'NT was detected. The experiments were performed in accordance with the decrease of blood pressure (40-50 mmHg). In this case the time of TF was prolonged in 4 time (LMWH) or 2 time (HMWH) comparatively with normal level. LMWH or HMWH combi-nations with DET or PZ administration led to normalization of 5'NT level in blood plasma. In arterio-venous shunt model it has been shown that the time of TF was prolonged in 3 time (saline), that was accompanied with the decrease of blood pressure (40-50 mmHg). In this case the time of TF was prolonged in 4 time (LMWH) or 2 time (HMWH) comparatively with normal level.

Results: The increase of anticoagulant and antithrombotic effects of LMWH or HMWH by pretreatment of DET or PZ were shown in both animal models of venous thrombosis. The degree of TF by Wessler model may be estimated as 3.7 (saline), 1.2 (LMWH), 1.8 (HMWH), 0.9-1.1 (DET+ LMWH or PZ+LMWH) and 1.1-1.3 (DET+ HMWH) or DET+ PZ+ HMWH). Besides, it has been shown that TF was accompanied with significant hypercoagulation of blood: 5'NT activity was increased in 2 time comparatively with normal level. LMWH or HMWH combi-nations with DET or PZ administration led to normalization of 5'NT level in blood plasma. Besides our results show that α–adrenoreceptor antagonists signifi-cantly improve antithrombotic effect of anticoagulant agents (LMWH and HMWH).

Therefore the combination of LMWH with selective and nonselective α–adrenoreceptor antagonists may be effective used for prevention of venous thrombosis development and thromboembolia.
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 (36.6%) 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 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 (50.8%). 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 tested every day until WBC >4.0×10^9/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 difference in age, gender and dose intensity of chemotherapy between the two groups (P>0.05). The average recovery time of the blood neutrophil granulocyte >0.5×10^9/L in experimental group and control group were respectively (6.52± 3.26) days versus (12.92± 4.75) days (P<0.05) and that of PLT >20×10^9/L was respectively (9.24± 3.88) days versus (13.15± 5.76) days (P<0.05). No UCBP transfusion-related side effects were found.

Summary/Conclusions: UCBP administration is safe as treatment for cytopenia and could promote blood cell recovery in patients with acute leukemia undergoing chemotherapy.

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 in Mafraq Hospital.

Methods: In 2011 the ordering practice at Mafraq Hospital, a designated Trauma Centre, had been evaluated. Data collected retrospectively over a one year period and a C:T ratio was adopted by the American Association of Blood Banks for determining the quantities for all various sub-specialties including Surgery, Internal Medicine, Pediatrics and Obstetrics and Gynecology. All procedures related to hospital transfusion practice have been 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-specialties. This practice of ordering was probably because of the fear that blood will not be available, if needed. Following implementation of control and continuous monitoring measures while establishing proper procedures such as transfusion guidelines, administration of blood and blood products and Maximum Surgical Ordering Practice, Mafraq blood bank, supported by the Transfusion and Tissue & Quality & Patient Safety Committees, achieved a great success in reducing C:T ratio <2 all through 2016 Figure 1. The reduction of C:T ratio had improved blood inventory control and reduced the workload of the blood bank staff. Because fewer units of cross-matched PRBC are being ordered, the blood bank has been able to decrease the number of expired units &reducing money loss Figure 1. The savings in technologist time is particularly significant since the blood bank is most of the time at a minimal staffing level.
Invasive hemostatic procedures were required in 20% of the whole series. In 2015–2016, we observed 948 hospitalized patients, who received 12,344 PC transfusions. Individual matching of PCUs was performed by cross-matching on the Galileo-Neo (Immucor) analyzer. Statistical processing was performed using the chi-squared test with Yates’ correction.

**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 PCUs was performed by cross-matching on the Galileo-Neo (Immucor) analyzer. Statistical processing was performed using the chi-squared test with Yates’ correction.

**Results:** Of 948 patients, 8 patients developed refractoriness to PC transfusions (12% of total patients). Out of them, 21 patients received 389 PC transfusions without individual matching. 86 patients had 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 recorded.

**Background:** According to European legislation (2002/98/EC, 2005/61/EC) as a requirement of hemovigilance system traceability (confirmation of final destination of blood components in hospitals) information should be kept for 30 years, improving the quality and safety of the transfusion process. Various methods are available from simple paper-based procedures to full electronic blood tracking systems. The ideal goal is to trace the final fate of 100% of the red blood cell (RBC) units, from donor to recipient and vice versa.

**Aims:** To check the ability to trace each individual unit from donor to recipient or disposal in our hospital.

**Methods:** To ensure compliance, the minimum traceability data set for retention is a mix of 1) Wards’ paper files (file of transfusions and/or patient records: 14/2 wards respectively). 2) HTL electronic records and paper records. The transfusion practitioner is responsible for the collection and maintenance of traceability data.

**Results:** During the year 2016, the number of RBC units transfused in our hospital was 2128. The traceability status of the transfused units is shown in the Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>Traceability of transfused units</th>
<th>Confirmable</th>
<th>Description</th>
<th>N</th>
<th>N total</th>
<th>2016?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed</td>
<td></td>
<td>Full match of data</td>
<td>2067/2128</td>
<td>97.9%</td>
<td>2016</td>
</tr>
<tr>
<td>Presumed</td>
<td></td>
<td>Wrong number of units in the ward</td>
<td>25/2128 (1.18%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presumed</td>
<td></td>
<td>No number of unit in the ward</td>
<td>2/7128 (0.03%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>No data in the ward*</td>
<td>29/2128 (1.36%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Patients’ data has been archived for long-term retention.*
ed, after matching to refractory patients the frequency was 0.002%. Before matching to refractory patients, the frequency of NHFR was (0.03%) (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Status</th>
<th>Patients</th>
<th>Transfusions</th>
<th>Post-transfusion reactions</th>
<th>% of all transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory</td>
<td>134</td>
<td>207</td>
<td>0.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-refractory</td>
<td>21</td>
<td>389</td>
<td>0.03</td>
<td>0.001</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 malaria may occur sublinically and has an high incidence of co-morbidities that could increase serum ferritin level, number of RBCT before and after starting the treatment.

Methods: We included 40 transfused patients treated with Deferasirox in three counties Hema-

ology Departments of North-West Romanian hospitals. Criteria of Deferasirox treatment: over 20 RBCT, serum ferritin level over 1000 microgl.

Results: We included 40 transfused patients treated with Deferasirox, age average 63. The diagnosis included sickeltsysplastic syndromes (most of patients), thalassemia, other anemias. Myelodysplastic patients were treated with low dose chemotherapy, epigenetic treatment, RBCT and Deferasirox. The serologic assessment was performed before starting Deferasirox treatment.

Background: Chelation therapy is recommended for transfused patients that have an elevated serum ferritin level (over 1000 microgl!), evidence of iron overload or received over 20 units of red blood cell transfusions (RBCT). Deferasirox showedefficacy and safety in maintaining or reducing body iron (assessed by liveriron concentration or serum ferritin).Iron chelation therapy was associated with hematopoiesis improvement in transfusion-dependent patients and interruption of Deferasirox treatment of transfusions dependent myelodysplastic patients produced loss of erythroid response.

Aims: Aim of the study: to assess the results of Deferasirox efficacy, side effects and to study if the number of RBCT decreased after starting Deferasirox.

Methods: We have done a retrospective, transversal study including all the adult politransfused patients treated with Deferasirox in three counties Hema-

ology Departments of North-West Romanian hospitals. Criteria of Deferasirox treatment: over 20 RBCT, serum ferritin level over 1000 microgl.

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).*

PB2234

EFFICACY 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 microgl!), evidence of iron overload or received over 20 units of red blood cell transfusions (RBCT). Deferasirox showedefficacy and safety in maintaining or reducing body iron (assessed by liveriron concentration or serum ferritin).Iron chelation therapy was associated with hematopoiesis improvement in transfusion-dependent patients and interruption of Deferasirox treatment of transfusions dependent myelodysplastic patients produced loss of erythroid response.

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Results: We included 40 transfused patients treated with Deferasirox, age average 63. The diagnosis included sickeltsysplastic syndromes (most of patients), thalassemia, other anemias. Myelodysplastic patients were treated with low dose chemotherapy, epigenetic treatment, RBCT and Deferasirox. The serologic assessment was performed before 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, 15 patients. In both groups the difference of RBCT means (before and after the start of the treat-

ment) 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 appli-
cation in clinical practice. This, coupled with blood shortages, the impossibility of transfusion in zero risk, the lack of evidence that transfusion may increase con-
sumption 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 restric-
tive transfusion (TR, Hb 7-9 g/dl) is not greater or lowerto the liberal trans-
fusion (TL, Hb 8-10 g/dl), keeping hemoglobin in safe levels for the patient.

Methods: A research was performed from October 1st, 2015 through Decem-
ber 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/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>100</td>
<td>8.1</td>
<td>9.3</td>
<td>1.0</td>
</tr>
<tr>
<td>LT</td>
<td>70</td>
<td>9.4</td>
<td>9.4</td>
<td>0.0</td>
</tr>
<tr>
<td>PWC</td>
<td>20</td>
<td>8.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>TPF</td>
<td>111</td>
<td>9.2</td>
<td>9.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

X: RBC transfused; 1:3

Hb Pre: Pre-transfusional haemoglobin; Hb Post: Post-transfusional haemoglobin; PWC: Patients without post transfusion Hbilevel; 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±4.0) and group (B): 52 polytransfused children (M:F 31:21; mean age:7±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.

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 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 6c and 6n regions. Of those, only 21 patients (42.6%) 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: Isohemaglutinins that develop against ABO blood group antigens are very important in transfusion and transplantation medicine. Today, 30-40% of allogeneic stem cell transplantations 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 Ig G and IgM titers in blood group A, anti-A IgG and IgM titers in blood group B and anti-A IgG and IgM 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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Figure 1.

Summary/Conclusions: Female individuals of blood bank donors participated in our study have higher isohemagglutinin titer values rather then 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 enchancement 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 titer values 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-
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.

**Aims:** To review our experience in the management of the primary TMA and to raise a series of questions that perhaps could improve the course of these pathologies.

**Methods:** We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age of 40-60 years. Only three cases had previous records of autoimmune diseases (MCTD, RA and HIV), all of which would eventually develop TTP. We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age of 40-60 years. Only three cases had previous records of autoimmune diseases (MCTD, RA and HIV), all of which would eventually develop TTP. We developed a technological scheme that involves fractionation plasma of blood in combinations of classical methods of precipitate protein and the two chromatographic steps: ion exchange and affinity chromatography.

**Results:** The process plasma fractionation is largest industry segment in manufacture of therapeutic concentrates of plasma proteins. We developed technological scheme that involves fractionation plasma of blood in combinations of classical methods of precipitate protein and the two chromatographic steps: ion exchange and affinity chromatography.

**Summary/Conclusions:** We developed technological scheme of plasma fractionation and reached a high degree of purification of coagulation FVIII.

**PB2239**

**PRIMARY TROMBOTIC MICROANGIOPATHIES. REVISION IN A CENTER OF THE LAST 8 YEARS**

T. Castaño1,*, S. Sanchez1, T. Arquero1, M. Yuste1, E. Askari1, P. Llamas1

1Fundación Jiménez Díaz, MADRID, Spain

**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.

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**Summary/Conclusions:** Thrombotic microangiopathies are a group of processes of enormous complexity, in addition to the low frequency with which they are usually present in our usual clinical practice requiring a large deployment of means to reach an early diagnosis and begin treatment as soon as possible given that the unfortunate prognosis of these patients. With this study we have raised a series of questions to improve the management of this type of diseases.

**PB2240**

**HAEMOVIGILANCE REPORTS OF ADVERSE BLOOD DONOR REACTION AMONG VOLUNTEER BLOOD DONORS IN TERTIARY CARE HOSPITAL IN KATHMANDU, NEPAL**

B. Nepal1, T. Castaño1,*, S. Sanchez1, T. Arquero1, M. Yuste1, E. Askari1, P. Llamas1

1Blood Bank, Grande International Hospital, Kathmandu, Nepal

**Background:** Voluntary blood donation is widely considered to be safe with very minimum chance of adverse reaction, which may occur during or after the end of phlebotomy procedure.

**Aims:** To identify and understand the complication of adverse donor reactions though the incidence of reactions of blood donation among blood donor in the tertiary care hospital in Nepal.

**Methods:** This is a prospective study done among voluntary blood donors at Grande International Hospital, Kathmandu, Nepal from February 2013 to March 2015. The outlines of reported and communicated adverse donor reaction were also collected after the blood donation from voluntary blood donors in different locations including outdoor and in-house blood donation drive.

**Results:** In the present study 6,955 whole blood donors were included, during the period of 2 years, 105 (1.50%) adverse donor reactions were reported. Majority 89(84.76%) of adverse donor reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylactic reactions were mild in nature such as; sweating; 27(25.72%), Light headedness; 19(18.09%), nausea and vomiting;

**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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Madrid, Spain, June 22 – 25, 2017
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Late Breaking Oral Session

LB2600
This abstract is part of the Presidential Symposium
NOVEL SMALL MOLECULE INHIBITORS CO-TARGETING CK1A AND P-TEFB DISRUPT SUPER-ENHANCERS AND ERADICATE ACUTE MYELOID LEUKEMIA IN A MOUSE MODEL
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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 CK1α robustly activates p53 (doi:10.1038/nature09673). However, with no selective CK1α inhibitors for in vivo use, the therapeutic value of CK1α inhibition in hematological malignancies cannot be validated.

Aims: To develop small molecule CK1α inhibitors and assess their effect in mouse models of human leukemia.

Methods: CK1α inhibitors were identified via cell-based screening based on p53 activation. We focused on a small class of pyrazole-pyrimidine scaffolds, which through extensive medicinal chemistry yielded derivatives with high affinity binding, validated by crystallography studies, potent CK1α inhibitory activity and a good pharmacokinetic profile. Anti-leukemic activity was assessed by oral treatment in mouse models of AML. MLL-AF9 and Bcr-Abl Blast Crisis Results: We first demonstrated the inhibitors’ anti-leukemic effect by single oral dose treatment, robustly inducing p53 activation and blast cell cytoreduction (Figure 1).

These inhibitors distinguished leukemic from normal hematopoietic stem cells: they did not affect normal hematopoietic CFUs, but eliminated leukemic CFUs at an IC50 < 9nM. We tested the long-term oral therapeutic effects of the inhibitors in MLL-AF9 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 HSPCs was demonstrated by transplanting leukemia-treated BM into lethally irradiated mice: all transplanted mice recovered and none showed any evidence of residual disease within 6 months. To elucidate the mechanisms by which the inhibitors distinguished leukemic from normal hematopoietic cells, we probed the kinase affinity of the inhibitors and further studied their signaling effects in vitro and in vivo. We found that CK1α inhibitors having potent anti-leukemia activity are distinguished from less active analogues by their capacity to co-target CKD9 and suppress the RNA Pol II elongation factor P-TEFb (CDK9-CyclinT1 complex). This property, validated by co-crystallography studies, enables the inhibitors to disrupt super-enhancers (SE), demonstrated by suppression of chromatin H3K27 acetylation and Brd4 association. As a result, transcription of SE-dependent major anti-apoptotic leukemia oncogenes including Mdm2, Bcl-2 and Mcl-1 was nearly abolished and inhibitor-treated leukemia cells underwent apoptosis. Strikingly, brief drug exposure (10mins in vitro; 2hrs in vivo) results in prolonged (24hrs) SE suppression. This unique property, which is at variance with the current occupancy-driven pharmaceutical paradigm, likely contributes to the dramatic therapeutic effect of co-targeting CK1α and P-TEFb in leukemia.

Summary/Conclusions: We developed a new class of small molecule inhibitors that co-target CK1α and P-TEFb. These inhibitors induce very rapid, robust activation of p53 in synergy with shutdown of leukemic super-enhancers, resulting in a lasting, powerful and specific anti-leukemic therapeutic effects in vivo, with cure potential.

Figure 1.

Single-donor inhibitor effects treated for 4hrs (EM Western blot and blood smear) and 16hrs (tissue record).

Figure 1. A-B: Inhibition of blast crisis in MLL-AF9 leukemic mice treated with MLL-AF9 inhibitor. C: Inhibition of blast crisis in Bcr-AblBlastic crisis mice treated with Bcr-Abl inhibitor. D: Cytoreduction of MLL-AF9 blast crisis treated with MLL-AF9 inhibitor. E: Cytoreduction of Bcr-Abl Blast Crisis treated with Bcr-Abl inhibitor.

P-TEF-B DISRUPT SUPER-ENHANCERS AND ERADICATE ACUTE MYELOID LEUKEMIA IN A MOUSE MODEL
Background: 1q (1q21 gain) is a common high risk subtype of multiple myeloma (MM), which drives MM progression, confers drug resistance, and correlates with inferior outcome. However, the molecular mechanism underlying the adverse prognostic roles of 1q remains largely unclear. Recently, 1q has been linked to hypoxia and resulting drug-resistant gene expression.

Aims: To understand the function and clinical significance of hypoxia-induced factor-1β (HIF-1β), a gene located in the 1q21 region, in 1q MM and hypoxic microenvironment.

Methods: The relationship between 1q or HIF-1β and Btz response or overall survival (OS) was analyzed in patients with newly-diagnosed MM (NDMM). Western blot and qPCR analyses were performed to determine expression of HIF-1β and other 1q21 genes in 1q+ MM cells, while no difference observed in CKS1B, a biomarker widely employed for 1q MM, or PSMB4 and MCL-1. Further, analysis of additional 40 NDMM patients revealed that HIF-1β mRNA level was significantly higher in MM patients, compared to normal donors (n=5, P < 0.005); analysis of the microarray database UAMS "Multiple Myeloma DataBase" (University of Arkansas) also showed that HIF-1β expression was higher with MM progression. In high, e.g., MF, MS, PR) vs low risk (e.g., CD1, CD2, HY, LB; P < 0.05) subtypes, or in w 1q vs w/o 1q (P < 0.001 for copy number ≥3), as well as cor-

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, =3, and <2 (P=0.300), respect-

Summary/Conclusions: Together, these findings argue that HIF-1β represents a potential biomarker for risk stratification and prognostic prediction of MM patients, especially those with high-risk cytogenetics such as 1q. They also suggest that HIF-1β might play a critical role in drug resistance related to microenvironmental factors (particularly hypoxia) and 1q21 gain, therefore serving as a potential therapeutic target for development of agents or therapy to overcome intrinsic and acquired drug resistance in MM.

Background: ARNT/HIF-1BETA LINKS POOR CLINICAL OUTCOME TO MICROENVIRON-

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**Summary/Conclusions:** Together, these findings argue that HIF-1β represents a potential biomarker for risk stratification and prognostic prediction of MM patients, especially those with high-risk cytogenetics such as 1q. They also suggest that HIF-1β might play a critical role in drug resistance related to microenvironmental factors (particularly hypoxia) and 1q21 gain, therefore serving as a potential therapeutic target for development of agents or therapy to overcome intrinsic and acquired drug resistance in MM.
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Background: CTL019 is an investigational chimeric antigen receptor (CAR) T-cell therapy with a high rate of durable complete responses (CRs) and a manageable safety profile in a previously reported single-center trial in adult patients (pts) with R/R DLBCL. Aims: Results of a planned interim analysis of a single-arm, open-label, multicenter, global phase 2 trial of CTL019 in pts ≥18 y with R/R DLBCL (JULIET; NCT02445248) are reported. Methods: Industry-manufactured CAR T cells were provided to pts at 27 centers on 4 continents using a global supply chain. Pts had received ≥2 lines of chemotherapy indicating that adipocyte-rich aged BM or pathologies enhancing adipocyte recruitment orchestrate T-ALL propagation. T-ALL derived from adipocyte–rich BM are associated with quiescence and decreased response to cell cycle dependent transcription programs. In addition, reprogrammed fibroblasts repopulate BM sites differently imprint dormancy and chemoresistance to T-ALL 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, TLX1/3 overexpression or SIL-TAL 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 CXCL12 - are key players in T-ALL development. Notch1, integrin LFA1/ICAM1 - and secreted factors - such as interleukin 7 and CXCL12 - are key players in 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 what extent these distinct niches imprint niche-specific features on T-ALL cells is not well understood. Aims: To 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 when they develop in lymph nodes (lymph nodes), thymus (thymus) and bone marrow (Bone marrow). T-ALL-geared 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 thymus share identical genomic abnormalities and functional disparities disappear in vivo and in prolonged in vitro assays. Importantly tail-derived T-ALL display a higher intrinsic resistance to cell cycle specific chemotherapies, i.e. vincristine sulfatate and cytarabine, but not to dexamethasone. T-ALL recovered from gonadal adipose tissue 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 imprint T-ALL propagation depending on their tissue origin. Lymph nodes, thymus and BM are different environments where T-ALL can develop with distinct properties that are 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.

LB2606

INDUCTION OF HEMOGENIC REPROGRAMMING IN HUMAN FIBROBLASTS

A. Gomes1, 2, C.-F. Pereira3, B. Chang1, 2, I. Kurochkina4, M. Daniel1, K. Law5, N. Satija6, A. Lachmann6, Z. Wang7, L. Ferreira8, A. Ma’ayan6, B. Chen5, D. Papatsenko9, I. R. Lemischka10, K. A. Moore11

Background: Hematopoietic stem cells (HSCs) are multipotent stem cells capable of sustaining all mature blood cells throughout life. During development, HSCs arise directly from specialized endothelial cells called hemogenic endothelial (HE) cells within the developing aorta-gonad-mesonephros (AGM) region, in a process termed endothelial-to-hematopoietic transition (EHT). However, despite extensive studies in various animal models, the genetic program driving human HSC emergence remains largely unknown. We have previously reported the generation of hemogenic precursor cells from mouse fibroblasts with the expression of transcription factors in vitro, namely GATA2 and Etv6. These TFs induce a dynamic, multi-stage hemogenic process that progresses through an endothelial-like, intermediate, recapitulating developmental hematopoiesis in vitro.

Aims: Here, to better understand the molecular events underlying human HE cell specification we expressed hemogenic TFs in human fibroblasts and mapped the TF binding sites at initial stages of reprogramming.

Methods: To determine the transcription factors binding sites we used Chro- matin Immunoprecipitation coupled with sequencing (ChIP-seq).

Results: We demonstrate that human fibroblasts can be reprogrammed into human HSC progenitors by the expression of GATA2. Gene expression data shows that they 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 reprogrammimg while GFP1B 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 promotors marked by H3K4me3, H3K4me1 and H3K27ac in the fibblast genome controlling the silencing of fibroblast genes while activating the hemogenic program.

Summary/Conclusions: Together, these findings uncover a collaborative TF interaction that specify a human hematogenic program and EHT. These findings shed light on the processes controlling human HSC specification and provide means to generate human reprogrammed HSCs at high efficiency for transplantation.
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